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## CONTENTS


Quantitative Inferences concerning the Genetic Effects of Radiation on Human Beings: *Robley D. Evans* ..... 299

Neomycin, a New Antibiotic Active against Streptomycin-Resistant Bacteria, including Tuberculosis Organisms: *Selman A. Waksman* and *Hubert A. Lechevalier* ..... 305

### Technical Papers

Electron Micrographic Observations of Tobacco Mosaic Virus in Crude, Undiluted Plant Juice: *Robley C. Williams* and *Russell L. Steere* ..... 308

Infrared Spectra and the Amide Linkage in a Native Globular Protein: *I. M. Klotz* and *Patricia Griswold* ..... 309

The Effect of Choline-Deficiency on Uterine Activity of Rats: *Charles P. Kraatz* and *Charles M. Gruber* ..... 310

A New Method of Inoculating the Maydeae with Smut Fungi: *Jack R. Wallin* ..... 312

The Semiquinone Radical of Tocopherol: *L. Michaelis* and *S. H. Wollman* ..... 313

Technique for Making Spreads of Omentum from Small Animals: *Joy Barnes Cross* ..... 314

Scanning Instrument for Quantitative One-Dimensional Paper Partition Chromatography: *L. S. Fosdick* and *R. Q. Blackwell* ..... 314

The Use of Ion Exchange for the Determination of Radioelements in Large Volumes of Urine: *Jack Schubert, Edwin R. Russell* and *Lawrence B. Farabee* ..... 316

### Book Reviews

Advances in food research: E. M. Mrak and George F. Stewart. Reviewed by *E. V. McCollum* ..... 318

A textbook of heat for upperclassmen: LeRoy D. Weld. Reviewed by *Mark W. Zemansky* ..... 318

### Scientific Book Register ..... 318

### News and Notes ..... 319



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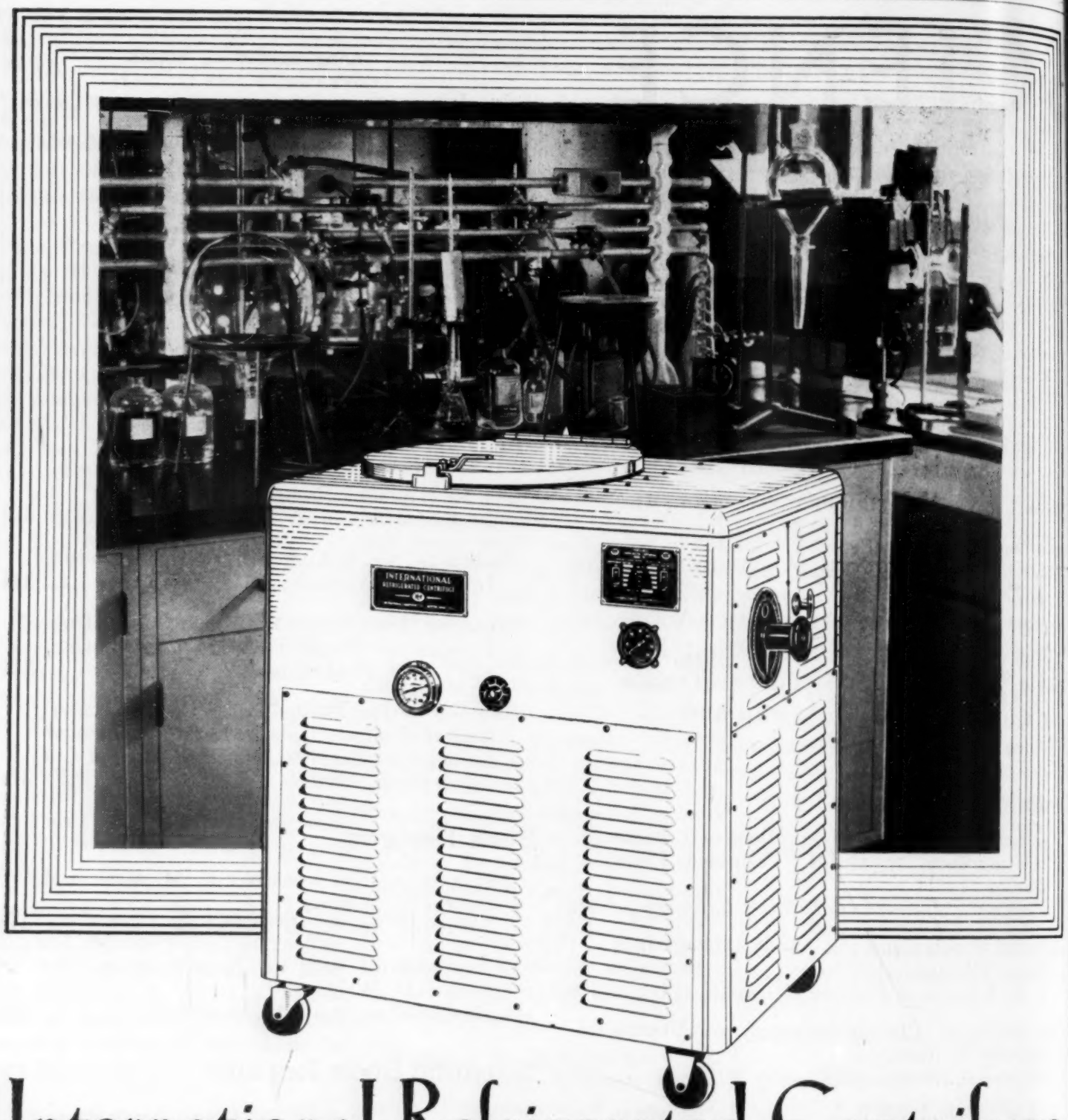


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# Quantitative Inferences concerning the Genetic Effects of Radiation on Human Beings

Robley D. Evans, *Department of Physics*
*Massachusetts Institute of Technology, Cambridge*

IN 1927 H. J. MULLER showed that the natural rate of appearance of new mutations in the fruit fly could be increased by irradiation with X-rays (*22*). Similarly, mutations have been produced in many other organisms, including plants such as corn, barley, and beans, by acute and by chronic irradiation. These observations have stimulated much speculation with regard to their applicability to mammalian forms and especially to man.

Lorenz and Heston (*20*) found that mice bred from five to six generations while continuously exposed, 24 hours a day, to 1.1 r and 0.11 r of radium gamma radiation[1] showed no damage to chromosomes, as evidenced by normal litter size and apparently normal life span. Deringer *et al.* (*7*) exposed mice chronically 8 or 24 hours a day to 8.8 r or 4.4 r. Total accumulated doses were for females 770 r and 880 r, and for males 1,100 r. No evidence for the production of visible genetic changes was obtained in the immediate offspring of mice thus irradiated. No evidence was found for the production of chromosome translocations in tests of the offspring of male mice exposed to a total dose of 1,100 r at the rate of 8.8 r given for 8 hours a day, nor in the offspring of female mice which received a total dose of 770 r at the rate of 8.8 r given 24 hours a day.

Hertwig (*14, 15*) demonstrated the production of four recessive gene mutations. Of these, two were produced in the offspring of male mice mated immediately after irradiation (irradiation of mature sperm), and resulted in retarded growth. The second two were produced in offspring of male mice mated after recovery from a period of temporary sterility following irradiation (irradiation of spermatogonia, or sperm-forming cells). They produced anemia and oligodactylism.

The acute dose in Hertwig's cases was 1,500 r of X-rays to the testes (*10*), the rest of the body being shielded to prevent death from whole body irradiation. The mutations were discovered by breeding the sons of the irradiated males to their own daughters. If the entire body had been irradiated at the dosage rate used locally in this experiment it would have received more than twice a lethal dose. Snell (*26*) found that about one-third of the progeny of mice whose testes were exposed to acute doses of about 600 r produced litters of reduced size. This condition of hereditary "semisterility" is believed to be caused by chromosome breakage and translocation (a class of *chromosomal* mutations [*10*]), not by *gene* mutation.

The importance of genetic changes in human beings justifies an attempt to extrapolate to man (*5, 8*) the abundant genetic data on the fruit fly, *Drosophila melanogaster,* and on plants. Catcheside especially has made commendable contributions toward quantitative estimations of the possible genetic effects on human beings of irradiation.

In *Drosophila melanogaster* which has only four pairs of chromosomes, genetic studies have led to the conclusion that the number of individual genes in each long chromosome is of the order of 1,000. We may take the set of chromosomes to include some 3,000 positions for genes. In man the total number of genes per gamete is unknown, but since there are 24 chromosomes, with a total length perhaps 10 to 12 times that of the four chromosomes of *Drosophila melanogaster,* the total number of genes in man is very likely greater than 5,000, and probably lies between $10^4$ and $10^5$. Recent estimates suggest a value of $(3 \pm 1) \times 10^4$ genes per gamete in man (*28*).

## Spontaneous Gene Mutations

In all organisms so far studied, there is a spontaneous gene mutation rate which has an average value such that the probability of mutation is of the order of $10^{-5}$ to $10^{-6}$ per gene per generation and appears to be independent of the average life span. Also in man the observed spontaneous mutation rate is about $10^{-5}$ per generation for hemophilia (*13*) and for epiloia (*11*).[2] Thus in long-lived organisms the gene

[1] One roentgen (r) of X radiation or of gamma radiation produces ionization in tissues to an average extent of about 1.6 ion-pairs per cubic micron, or $1.6 \times 10^{12}$ ion-pairs per gram of tissue.

[2] These two diseases happen to be well suited to the calculation of mutation rates, and no reason is known for suspecting that the rates found should be any higher or lower than for genes whose mutation rates are now unknown. It is, of course, possible that these measured mutation rates in man are automatically selected samples of genes which are relatively more mutable than the average of all genes in man. The decision must obviously await the accumulation of quantitative data on other mutations in man. It is interesting to note that a value of about $10^{-3}$ per gene per generation was found (*23*) in the Italian population of Rochester, New York, for the mutation causing thalassemia, a disease which appears to be found predominantly in persons of Mediterranean derivation.

material appears to be somewhat more stable, per unit of time. The range of numerical values is easily explained on quantum mechanical grounds (*25*). Apparently, the chemical activation energy, for an isomeric change in the configuration of the molecule or molecules comprising the gene, is slightly greater for the genes of long-lived animals. Small differences in this activation energy (which is of the order of 1 to 2 electron volts) can account for differences of several millionfold in the mean life of a gene configuration. Thus the observation that the average spontaneous mutation rate is of the order of $10^{-5}$ per gene per generation, regardless of life span, is experimentally and theoretically acceptable.

If there are $N$ genes per gamete, and if the average probability of a spontaneous recessive mutation is $a = 10^{-5}$ per gene per generation, then $aN$ is the mean number of new recessive mutations per gamete per generation. It has been estimated (*5*) that seriously deleterious and lethal mutations constitute perhaps one-quarter or less of the spontaneous mutations, and that minor recessive mutations predominate. In the long run, the extremely unfavorable mutations leading to hereditary abnormalities are almost wiped out by natural selection. The recessive mutations become distributed in the population of succeeding generations, and appear in an individual only if both parents carry the same recessive mutation.

The generation time for man is about 25 years, or 40 generations in 1,000 years. Thus there has been a long accumulation of recessive mutations, and each individual carries far more mutated genes than have arisen through mutation in his own generation. Because of relatively recent social changes such as the industrial revolution, the human population is not in genetic equilibrium (*12*). The persistence (*12, 16*) of any particular recessive mutation in the population will depend on the degree of inbreeding in the population, loss of particularly unfit mutants by natural selection, loss by reverse mutations or second mutations of the same gene, and interbreeding of semi-isolated population groups. Because genetic equilibrium between mutation and selection cannot be assumed for the slightly deleterious mutants, it seems reasonable to assume, somewhat arbitrarily, that at least $m = 50$ times as many recessive mutations have been accumulated in the population as are produced by spontaneous mutation in each generation. Then if $aN$ is the spontaneous mutation rate per generation, and $m$ is the accumulation factor, the average number of recessive mutant genes per germ cell of each member of the population may be written as $n = m \cdot aN$. Assuming, tentatively, that $m = 50$, we have $n = 50 \cdot aN$.

If a particular sperm cell contains $n_1$ recessive mutations distributed randomly among $N$ genes, then the chance that a particular designated gene carries a recessive modification is $n_1/N$. Similarly if an ovum carries $n_2$ recessives, the chance that the same designated gene carries a recessive mutation is $n_2/N$. The chance of coincidence, in the union of these two gametes, is then $(n_1/N)(n_2/N)$ for the particular designated gene. Because there are $N$ genes altogether in each gamete, the chance of observing a coincidence of any two recessives in any of these $N$ genes is then $(n_1/N)(n_2/N)N = n_1n_2/N$. Then the statistical chance of the occurrence of a hereditary abnormality or anomaly due to the coincidence of two recessive mutations is $C_0 = n_1n_2/N$. If we assume $n_1 = n_2 = maN = (50 \times 10^{-5})N$, and that $N$ is as large as $10^5$, then $C_0 = (2.5 \times 10^{-7})N = 0.025$ is the estimated fraction of all births[3] in which some sort of recessive modification may be expected to appear visibly in the progeny.

## Radiation-induced Gene Mutations

The mutations that can be induced by X-rays, gamma-rays, or ultraviolet light in a variety of organisms are not novel types of changes, but are similar to the mutations that occur spontaneously. The number of mutations produced is simply increased by the irradiation. Radiation-induced recessive mutations can be identical with spontaneous mutations in an unirradiated individual. A gene mutation that has occurred spontaneously or that has been produced by radiation (e.g., $a \rightarrow a'$), can return to the original, or "wild type" ($a' \rightarrow a$), spontaneously or as a result of irradiation of the progeny, although the statistical chance of this happening is low.

Although most of the spontaneous and the radiation-induced mutations are undesirable, a few are advantageous. For example, the present high commercial yields of penicillin are due in part to the exclusive use of high yield strains of *Penicillium*, selected from a large number of radiation-induced mutants of the naturally occurring parent mold (*1–3, 6, 17, 24*). Of course, this high yield is beneficial to *us*, but it has not been proved rigorously that it is beneficial to the *Penicillium*.

In *Drosophila melanogaster*, gene mutations in the X-chromosome of mature sperm have (*5*) an average induction rate of about $b_X = 3 \times 10^{-8}$ per gene per roentgen. The mutation induction rate at a particular gene locus varies by as much as a factor of 5 in either direction from this average value. Most of the

[3] It has been estimated that 22 percent of all human conceptions terminate in nonviable offspring (*28*). This includes all causes of death of the foetus, of which an undetermined but presumably small fraction are genetic causes.

measurements have been accomplished by the use of genetic techniques especially applicable to the observation of sex-linked lethals, but enough has been done on sex-linked visible recessives to suggest that the results are similar. Presumably about the same average numerical value will also apply to gene mutations in the other chromosomes, although present genetic techniques have led to numerical results only for genes located along the X-chromosome in irradiated sperm.

There are measurable differences in the mutation induction rate in sperm of *Drosophila melanogaster* which have been irradiated at various stages in the process of spermatogenesis. When immature sperm are irradiated at the early spermatogonial stage, the number of visible sex-linked mutations eventually produced is only about one-half as great as is found following the same irradiation dose given to mature sperm. The effective ratio of the radiation sensitivity of immature to mature sperm is even smaller (ratio of about 1:5) for sex-linked lethal mutations. These effects probably arise because many of the altered spermatogonia, or immature sperm, fail to survive through the process of spermatogenesis and hence never become available as mature sperm (*10*).

At radiation rates of 0.6 r/hr and greater, the number of sex-linked mutations induced in mature sperm of *Drosophila melanogaster* appears to be independent of dose rate (*19*), or quantum energy, and to be linear (*27*) with total irradiation dosage from 25 r to at least 1,000 r. The assumption is usually made that this constant mutation induction rate per roentgen will hold even at very low dosage rates. Recently, however, experiments have been completed (*4*) at the very low dosage rate of 2.5 r/day. Mature sperm of the Canton Special strain of *Drosophila melanogaster,* stored in the spermathecae of females of the Muller-5 strain, were irradiated with radium gamma-rays at a rate of 2.5 r/day for 21 days, at the end of which time the sperm had accumulated a total dose of 52.5 r. The progeny resulting from subsequent fertilization by these aged and irradiated sperm showed an unexpectedly small number of mutations, and indeed was not significantly different statistically from that of unirradiated controls. The number of sperm tested was such that the probability is less than one percent that the result is merely a chance deviation from the rule of proportionality between total dosage and the number of induced mutations. The possibility therefore exists that the genetic effects per roentgen may be less at very low dosage rates than the effects previously observed at higher dosage rates, where repair or recovery of the gene material has not been observed. Further experiments at low dosage rates are needed to answer this question, since it has an important bearing on the "point-hit theory" of radiation effects and on the extrapolation of data to the case of men who are occupationally exposed to radiation at very low dosage rates.

In more recent studies (*27*) of the Canton Special strain of *Drosophila melanogaster* it required a dose of about 50 r to mature sperm in order to double the number of new sex-linked visible and lethal mutations. In these observations the spontaneous mutation rate for sex-linked lethals is 0.0010, while the induced rate is 0.00002 per r. Writing $N_X$ for the number of gene loci on the X-chromosome, we have $a_X N_X / b_X N_X = 0.0010/0.00002 = 50$ r to double the spontaneous mutation rate. If $N_X$ is about 1,000, the spontaneous mutation rate would be about $a_X = 10^{-6}$ per gene per generation, and the induced mutation rate would be about $b_X = 2 \times 10^{-8}$ per gene per roentgen, for this strain under the particular cultural conditions of this experiment.

Quantitative observations of the rate of induction of mutations by radiation have been made in a variety of organisms, including *Drosophila,* tobacco mosaic virus, and bacteria. The results are remarkably similar. Because such widely different organisms as *Drosophila,* virus, and bacteria exhibit substantially the same rate of induced mutation per roentgen, it is usually assumed that about the same average numerical value, $b = 3 \times 10^{-8}$ per gene per roentgen, may also hold for induced mutations in mature germ cells in man.

In man, mature sperm have a fertile life span of about seven weeks (*10*) but the relative sensitivity of sperm and eggs at various developmental stages is unknown. We may represent the time average of the relative radiation sensitivity of immature sperm and eggs in man by $k$, as compared with the relative sensitivity $k = 1$ for mature gametes. For low level radiation continued over a time which is long compared with seven weeks, $k$ may be significantly less than unity for the male, but substantially unity for the female. If these factors could be evaluated numerically for man, then in the equations and computations which are to follow one would replace the radiation sensitivity, $b$ per gene per roentgen, by the smaller quantities $k_1 b$ for males and $k_2 b$ for females. Because of the large uncertainty in the currently available numerical estimates of $k$, we would hardly be justified in carrying this refinement into the calculations. So in what follows we shall use the maximum values $k_1 = k_2 = 1$, thereby assuming that immature sperm and eggs have as great a radiation sensitivity as mature gametes. We will therefore overestimate the genetic effects of chronic irradiation, insofar as this particular factor is concerned.

### Ratio of Induced to Spontaneous Mutations

It is interesting to note that cosmic radiation and local gamma radiation, totaling about 0.3 mr per day, are entirely inadequate to account for the natural spontaneous gene mutation rate. Even in a long-lived organism such as man, the dosage from natural sources of radiation amounts to only about 0.1 r per year, less than 3 r between birth and the average childbearing age. Such a small amount of radiation would give only about $3b = 9 \times 10^{-8}$ mutations per gene per generation, whereas the few observed spontaneous human rates of $a = 10^{-5}$ per gene per generation are more than 100 times as large.

We see that a total dosage of the order of $aN/kbN = 10^{-5}/3 \times 10^{-8} = 300$ r per generation is required to bring the induced rate up to equality with the natural rate of mutation per generation. Thus an average dosage of about 300 r per individual per generation would be required to double the natural rate of appearance of new gene mutations per generation. If we chose a lower numerical value for the spontaneous mutation rate, say $a = 3 \times 10^{-6}$, the rate-doubling dose $a/b$ would become about 100 r, provided $k = 1$.

A radiation worker whose whole-body dosage, and hence gonadal dosage, is limited to 0.1 r per working day, as is now the practice in the United States, could at most receive 0.5 r in a five-day week, or 25 r a year, or 250 r in a 10-year working period before childbearing. Actually, the daily average radiation dose per worker will fall far below this maximum because on many days an individual's dose will be much less than the maximum permitted value of 0.1 r. For example, the average daily dose at installations like the Hanford Engineer Works and the Oak Ridge National Laboratory is about 0.005 r. Only rarely does any individual receive the maximum 0.1 r dose. Thus the average 10-year dose, at 0.005 r per day, is only about 20 r for these groups.

In the notation introduced earlier, we may write that the number $n_1$ of recessive mutants transmitted in an individual's germ cells will be the sum of the accumulated naturally occurring mutants, $maN$, plus the induced mutants, $kbDN$, where $D$ is the dose in roentgens which this individual receives before he has children. Thus:

$$n_1 = maN + kbDN$$

Writing $n_0 = maN$ for the naturally occurring mutants, and $f_1$ for the ratio of the new total $n_1$ to the unirradiated total $n_0$, we have:

$$f_1 = n_1/n_0 = (maN + kbDN)/maN = 1 + \frac{kbD}{ma}$$

If the numerical values developed earlier turn out to be approximately correct for man, then with $b = 3 \times 10^{-8}$ per gene per r, $a = 10^{-5}$ per gene per generation, an accumulation factor of $m = 50$, and the relative radiation sensitivity of immature to mature gametes $k = 1$, we have:

$$f_1 = 1 + (6 \times 10^{-5})D$$

Thus a radiation worker who received $D = 250$ r before having children will have $f_1 = 1 + 0.015$, and $n_1 = 1.015n_0$, or 1.5 percent more mutants than if unirradiated. If he then has children by an unrelated and unirradiated spouse ($n_2 = n_0$), the statistical chance of an inherited anomaly due to recessive gene mutations is: $C = n_1n_2/N = (1.015n_0)n_0/N = 1.015C_0$, or only 1.5 percent greater than the normal chance $C_0$.

The occurrence of inherited anomalies in the first generation offspring due to *dominant* mutations induced in one parent by radiation is more difficult to estimate numerically because of the lack of numerical data, but may be the more important hazard. Here the accumulation factor, $m'$, will be smaller and will depend more strongly on the degree of unfitness conferred by the particular mutant. The degree of dominance (or "penetrance") of the mutant will affect its visibility as well as its accumulation. For the sake of estimating an order of magnitude, we might make some rough assumptions. If the ratio of induced to spontaneous dominant mutations, $(b'/a')$ is the same as $(b/a)$ for recessive mutations, and if the penetrance is taken at its maximum value of unity, and the fitness taken such that the accumulation is about $m' = 3$, then the ratio, $f'$ of induced to spontaneous dominant mutant genes per gamete would be very approximately:

$$f' = (m'a'N + kb'DN)/m'a'N$$
$$= 1 + kb'D/m'a' \approx 1 + k(b/a)D/m'$$
$$1 + 1 \times (3 \times 10^{-8}/10^{-5})D/3 \approx 1 + 10^{-3}D,$$

or about 1.25 for $D = 250$ r. That is, if the penetrance is unity, then the statistical chance of the appearance of a dominant anomaly in the first generation offspring would be increased by about 25 percent over the chance of the same anomaly's occurring spontaneously. It is interesting to note that the medical literature already contains reports of over 2,250 cases of women whose ovaries were treated with X-rays (mostly 50 to 100 r, for various gynecological conditions) and that no anomalies definitely attributable to radiation have been observed in their subsequent offspring (*9, 10, 18, 21*). The offspring would show dominants, if present, and male offspring would show sex-linked recessives, if present. However, other recessives would not be visible in the first generation offspring.

The natural rate of congenital anomalies $C_0$ is small, and is already influenced by such factors as

nutrition, consanguineous marriages, and socio-economic shifts. Therefore, many eugenists and geneticists feel that no significant eugenic harm would be done by the additional factor of radiation, provided that radiation doses to the general population were confined to values that would not more than double $C_0$ within the foreseeable future. Whereas we see that the effects in the first generation progeny are small, we must also consider the accumulated effects of recessive mutations over, say, 2,000 years. Because the induced mutations are similar to the natural mutations, and because genetic equilibrium cannot be assumed, we should use the same accumulation factor, $m$, for the ratio of the accumulated induced mutations present after many generations to the new induced mutations per generation. Dealing now with population averages, and assuming equal average irradiation for both sexes, we have after many generations:

$$\begin{aligned} n_1 = n_2 &= maN + mkbD_1N \\ &= n_0(1 + kbD_1/a) \end{aligned}$$

where $D_1$ is the average, over all individuals of both sexes, of the number of roentgens to the gonads per generation up to the end of the childbearing period. If we require that the congenital anomalies be no more than doubled, then

$$2 = \frac{C}{C_0} = \frac{\frac{n_1 n_2}{N}}{\frac{n_0 n_0}{N}} = \left(1 + \frac{kbD_1}{a}\right)^2$$

hence: $$1 + kbD_1/a = \sqrt{2}.$$

It is to be noted that this general result, for the condition after many generations, is independent of the accumulation factor $m$, and that the genetically permissible average dose $D_1$ depends only on the average spontaneous mutation rate $a$, and the average induced mutation rate $b$. Again assuming $a = 10^{-5}$ per gene per generation, $k = 1$, and $b = 3 \times 10^{-8}$ per gene per roentgen, we find:

$$\begin{aligned} D_1 &= (\sqrt{2} - 1)(a/kb) \\ &= 0.414 \times 10^{-5}/3 \times 10^{-8} \\ &= 140 \text{ roentgens.} \end{aligned}$$

Since the dose $D_1$ deals with population averages (both men and women) and is the mean dose per head, $D_1 = 140$ r could be realized by 100 percent of the population receiving 140 r, or 50 percent receiving 280 r, or 10 percent receiving 1400 r, etc. If, for example, as many as five percent of the total population received an average dose of 280 r before childbearing, then the average dose to the entire population would be $0.05 \times 280 = 14$ r, and the eventual fractional increase (after, say, 2,000 years) in congenital anomalies due to radiation could be expected to be about:

$$\begin{aligned} C/C_0 &= (1 + 3 \times 10^{-8} \times 14/10^{-5})^2 \\ &= (1 + 0.041)^2 \\ &= 1.08 \end{aligned}$$

or only eight percent greater than the spontaneous rate in a similar unirradiated population. If we assume a smaller value for the spontaneous mutation rate, say, $a = 3 \times 10^{-6}$, then for $C/C_0 = 2$, the average dose per head per generation becomes $D_1 = 42$ r, while an average dose of $D_1 = 14$ r would give only $C/C_0 = (1 + 0.14)^2 = 1.30$, or 30 percent greater than the spontaneous rate in a similar unirradiated population.

*Conventional equilibrium theory.* In a statistically large population, it is well known (e.g., Hogben, *16*, p. 195) that if $s$ is the fractional reduction of net fertility in individuals who are homozygous with respect to a particular recessive mutant, then when genetic equilibrium between selection pressure and mutation pressure is reached, the gene frequency of this recessive will be $\sqrt{a/s}$. Thus in our notation, $ma = \sqrt{a/s}$, or $m = \sqrt{1/as}$ for spontaneous mutations, while $ma + mbkD = \sqrt{a + bkD)/s}$ for the sum of the spontaneous and the induced mutations. However, if the selection coefficient, $s$, is small, as is probable for many of the minor recessive mutations, then constant conditions over a period of many thousand generations are required before genetic equilibrium with respect to such genes is closely approached (e.g., Hogben, *16*, p. 143). Even so, this standard treatment in the existing mathematical theory of genetics neglects reverse mutations or second mutations of the same gene, inbreeding, statistical fluctuations in partially isolated population groups of finite size (*29*), and population shifts which result in interbreeding between population groups.

It may be worth while to note what could be deduced for human populations, if it were permissible to assume genetic equilibrium. The effective accumulation factor $m = 1/\sqrt{as}$ for minor recessive mutations might then be of the order of magnitude of 1,000 or more after many thousand generations of constant conditions. This would reduce our estimate of the first generation effects by a factor of $1{,}000/50 = 20$ or more, and suggests that our estimate of $m = 50$ for the nonequilibrium case may even be unduly conservative. After thousands of generations of exposure to $D_1$r per generation, the new equilibrium mutant gene frequency per gene locus would be $\sqrt{(a + kbD_1/s}$, and the ratio $C/C_0$ of birth anomalies in irradiated and unirradiated populations would become $(a + kbD_1)/a = (1 + kbD_1/a)$, instead of the square of this quantity, as derived for the nonequilibrium case. Thus again, the numerical estimates made on the nonequilibrium,

or square-law model, appear to lie well on the conservative side.

Many simplifications of known genetic facts have had to be made in arriving at these numerical values but the analytical form and the general order of magnitude of the results should not have been altered thereby. Certainly the gradual accretion of knowledge in cytogenetics will permit future refinements in such calculations. Although one cannot be dogmatic where extrapolations of data are involved, it does seem highly improbable that any detectable increase in hereditary abnormalities will result, even after many generations, from daily radiation doses up to 0.1 r per day given to a small fraction of the population.

To review the genetic concepts and experiments most pertinent to the question of mutations induced by radiation: For a variety of organisms, the probability of a spontaneous gene mutation is of the order of $10^{-5}$ to $10^{-6}$ per gene per generation, and is substantially independent of the life span. Experiments on several types of organisms have shown that irradiation can produce gene mutations. These induced mutations are not novel types, but appear to be entirely similar to those which occur spontaneously. When the irradiation is carried out at a rate of 0.6 roentgens per hour, or higher, the average probability of inducing a gene mutation is about $3 \times 10^{-8}$ per gene per roentgen. The spontaneous mutation rate is therefore very much higher than could be expected as a result of the cosmic radiation and local gamma radiation (0.3 mr/day) and is therefore due to other causes than irradiation. When the irradiation of experimental organisms is carried out at a low rate (order of 1 to 8 roentgens per day) no induced mutations have yet been observed in the organisms studied (fruit fly, mouse). This suggests that the effective average radiation sensitivity of immature sperm and eggs may be less than the sensitivity of mature sperm and eggs. Dominant mutations may become visible in the first generation offspring. Recessive mutations may appear only in homozygous individuals of a later generation, and therefore recessive mutations accumulate in the population as a result of both spontaneous and induced mutations. From the appropriate mathematical theory, and the experimental data now available, it seems safe enough to conclude that no detectable increase in hereditary abnormalities is likely to result, even after many generations, if a small fraction of the population receives daily radiation doses up to 0.1 roentgen per day.

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# Neomycin, a New Antibiotic Active against Streptomycin-Resistant Bacteria, including Tuberculosis Organisms[1]

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THE ISOLATION OF STREPTOMYCIN from two strains of an actinomyces identified as *Streptomyces griseus,* was reported (*8*) from this laboratory five years ago. This was later followed by announcements that this antibiotic is highly effective against the tuberculosis organism (*9*), that it is active *in vivo* against a variety of pathogenic bacteria, and that it is not very toxic to animals (*7*). The ability of streptomycin to control experimental tuberculosis was soon established (*2*). Within less than a year after the isolation of streptomycin had been reported, its effectiveness upon certain forms of human tuberculosis was demonstrated (*3*). Thus, for the first time in the history of human tuberculosis, a drug was found which could be used in the treatment of tuberculosis by means of chemotherapeutic agents and which pointed to the possibility of finally eradicating the "white plague" of man.

Streptomycin has, however, certain important limitations, chief among which are (a) its neurotoxic potentialities, and (b) the development of resistance among the infectious organisms upon its prolonged administration. Recent experiments (*4*) seem to indicate that the toxic reactions can apparently be overcome, at least partly, by the use of dihydrostreptomycin, a reduced form of streptomycin. The problem of development of resistance remains, therefore, as the major limitation of this antibiotic, especially in the treatment of tuberculosis.

The possibility of isolating or synthesizing new compounds that would be effective against the streptomycin-resistant strains of bacteria suggested itself. Various reports indicated that certain synthetic compounds such as the sulfones and para-aminosalicylic acid, and various antibiotics such as different forms of streptothricin (*5*) and aureomycin (*1*) might prove to possess such properties. Although the results obtained thus far in some of the preliminary investigations with these substances are highly encouraging, they still appear to be inconclusive. We do not yet know whether or not any of these would serve to replace streptomycin, completely or partly, or to supplement it on more than a very limited scale. They may not be sufficiently active against the bacteria, or they may be toxic to animals and man, or they may lack stability, or have some other limitations.

In connection with the studies on the production of antibiotic substances by microorganisms, carried out in our own laboratories since 1939, many thousands of cultures, mostly actinomycetes belonging to the genus *Streptomyces,* were isolated from soils, composts, peats, and other natural substrates and tested for their activity against different bacteria; emphasis has been laid, in recent years, upon the mycobacteria. Surprisingly enough, a large number of the organisms thus isolated were found to possess considerable activity against *Mycobacterium tuberculosis.* Unfortunately, only a very few of the antibiotics produced by these organisms proved to be suitable chemotherapeutic agents, because of various limitations, some of which were just mentioned. The fact that numerous strains and species of *Streptomyces* possess the capacity to inhibit the growth of mycobacteria, however, and even to produce active antituberculosis compounds, and the fact that one of these, streptomycin, already had found practical application as a chemotherapeutic agent, offered the hope that sooner or later other substances would be found that might be even better. In search for new compounds, particular attention was paid to those that would be effective against streptomycin-resistant bacteria, notably against the streptomycin-resistant strains of *M. tuberculosis.* The discovery of such an agent, designated as *neomycin,* is reported here.

The organism producing neomycin was isolated from the soil. It is somewhat related to a culture isolated from the soil in 1915 by Waksman and Curtis (*10*) and designated as *Actinomyces fradii* (now listed in Bergey's Manual as *Streptomyces fradiae*).

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[2] The authors wish to express their sincere appreciation to Dr. A. Swart, Miss D. Hutchison, Mr. D. Harris, Mr. W. Iverson, and Mr. E. Katz of this laboratory, for assisting with some of the investigations reported here; also to Mr. J. Frankel of this laboratory, and Mr. O. Graessle, of the Merck Institute of Therapeutic Research, for assisting with the *in vivo* studies.

When the newly isolated culture was grown in various media containing a source of nitrogen (peptone, meat extract), a carbohydrate (glucose), and salt (NaCl),

TABLE 1

ANTIBACTERIAL ACTIVITY OF CULTURE FILTRATE CONTAINING NEOMYCIN

| Test organism | Units of activity in 1 ml of broth | |
|---|---|---|
| | Incubation* 3 days | Incubation* 5 days |
| *Escherichia coli* | 60 | 100 |
| *Bacillus subtilis* | 350 | 1,000 |
| *B. mycoides* | 330 | 400 |
| *Staphylococcus aureus* | 330 | 330 |
| *Mycobacterium* 607 | 330 | 1,000 |
| *M. ranae* | 330 | 1,000 |
| *M. avium* | 200 | 600 |
| *M. phlei* | 330 | 1,000 |

* Shaken cultures incubated at 28° C.

it was found to produce neomycin under both stationary and submerged conditions of culture. The antibiotic can easily be removed from the culture medium and concentrated by the methods of adsorption and elution which have been developed for the isolation of streptothricin and streptomycin from their respective culture media.

Neomycin is a basic compound, most active at an alkaline reaction. It is soluble in water and insoluble in organic solvents. It is thermostable. It is active against numerous Gram-positive and Gram-negative bacteria, especially mycobacteria, but not against fungi. This is brought out in Tables 1 and 2. The antibiotic spectrum of the crude neomycin is quite distinct from that of streptomycin, on the one hand, and of streptothricin, on the other.

TABLE 3

EFFECT OF NEOMYCIN UPON THE GROWTH OF DIFFERENT STRAINS OF *M. tuberculosis* IN DUBOS MEDIUM*

| Test organism | Growth inhibition units/ml |
|---|---|
| *M. avium*† | 4.0 |
| *M. avium* R†‡ | 4.0 |
| *M. tuberculosis* H37Rv† | 0.1 |
| *M. tuberculosis* H37RvR† | 0.2 |
| *M. tuberculosis* H37Rv | 0.5–1.0 |
| *M. tuberculosis* H37RvR | 1.0 |
| *Mycrobacterium* 607 | 0.1 |
| *Mycrobacterium* 607R | 0.25 |

* Incubation at 37°/C for 14 days.
† This particular test was made in another laboratory.
‡ R = streptomycin-resistant strain.

Neomycin preparations were found to possess several desirable properties, which fully justified a more detailed study of this antibiotic: (1) similar activity against both streptomycin-sensitive and streptomycin-resistant bacteria; (2) considerable activity (in some cases greater activity than streptomycin) against various forms of *M. tuberculosis* and other mycobacteria (see Table 3); (3) limited toxicity to animals or none; (4) activity against various bacteria *in vivo*, including Gram-positive and Gram-negative organisms and against both streptomycin-sensitive and streptomycin-resistant organisms (see Table 4); (5) lack of

TABLE 2

ANTIBIOTIC SPECTRUM OF CRUDE NEOMYCIN, AS COMPARED TO THAT OF CRUDE STREPTOMYCIN AND STREPTOTHRICIN

| Test organism | Dilution units per gm required to inhibit growth of test organisms | | |
|---|---|---|---|
| | Neomycin | Streptomycin* | Streptothricin* |
| *B. subtilis* | 150,000–750,000 | 125,000 | 125,000 |
| *B. mycoides* | 20,000–150,000 | 20,000 | 1,000 |
| *B. cereus* | 20,000– 60,000 | 30,000 | 1,000 |
| *S. aureus* | 100,000–250,000 | 15,000 | 50,000 |
| *S. lutea* | 10,000 | 100,000 | 37,500 |
| *E. coli* SS† | 25,000 | 25,000 | 25,000 |
| *E. coli* RS‡ | 20,000 | 0 | Active§ |
| *Ps. aeruginosa* | 2,500 | 1,000 | 1,000 |
| *Pr. vulgaris* | 25,000 | 10,000 | 12,500 |
| Bodenheimer's organism | 15,000 | 0 | Active§ |
| *Serratia marcescens* | 20,000 | 25,000 | 1,200 |
| *Mycobacterium* 607 | 80,000–250,000 | Active§ | Active§ |
| *Mycobacterium* 607RS | 50,000–150,000 | Inactive | Active§ |
| *Trichophyton mentagrophytes* | < 300 | < 300 | Active§ |
| *Candida albicans* | < 300 | < 300 | Active§ |

* From Shatz, Bugie and Waksman (8).
† SS = streptomycin-sensitive.
‡ RS = streptomycin-resistant.
§ Active = activity established in other reports.

resistance against neomycin among the organisms sensitive to it, or only limited development of such resistance.

TABLE 4

COMPARATIVE ACTIVITIES OF NEOMYCIN AND STREPTOMYCIN *in Vivo* UPON STREPTOMYCIN-SENSITIVE AND -RESISTANT BACTERIA

| No. of animals or embryos | Treatment | Dilution of bacterial culture | Surviving mice or egg embryos in days | | | Percent survival |
|---|---|---|---|---|---|---|
| | | | 2 | 5 | 10 | |
| | *Staphylococcus aureus*—streptomycin-sensitive* | | | | | |
| 5 | Control | $10^{-3}$ | 0 | 0 | 0 | 0 |
| 5 | Control | $10^{-5}$ | 1 | 1 | 1 | 20 |
| 5 | Streptomycin, 25 μg | $10^{-3}$ | 5 | 5 | 5 | 100 |
| 5 | Streptomycin, 100 μg | $10^{-3}$ | 5 | 5 | 5 | 100 |
| 3 | Neomycin, 50 units | $10^{-3}$ | 3 | 3 | 3 | 100 |
| 3 | Neomycin, 200 units | $10^{-3}$ | 3 | 3 | 3 | 100 |
| | *Salmonella schottmulleri*—streptomycin-resistant* | | | | | |
| 5 | Control | $10^{-3}$ | 0 | 0 | 0 | 0 |
| 5 | Control | $10^{-4}$ | 4 | 2 | 0 | 0 |
| 5 | Streptomycin, 100 μg | $10^{-3}$ | 2 | 0 | 0 | 0 |
| 5 | Streptomycin, 5000 μg | $10^{-3}$ | 1 | 1 | 1 | 20 |
| 5 | Neomycin, 100 units | $10^{-3}$ | 5 | 5 | 5 | 100 |
| 5 | Neomycin, 200 units | $10^{-3}$ | 5 | 5 | 5 | 100 |
| | *Salmonella pullorum*—streptomycin-sensitive | | | | | |
| 10† | Control | $10^{-2}$ | 4 | 0 | 0 | 0 |
| 10 | Control | $10^{-4}$ | 6 | 0 | 0 | 0 |
| 10 | Neomycin, 100 units | $10^{-2}$ | 5 | 5 | 5 | 50 |
| 10 | Neomycin, 100 units | $10^{-4}$ | 9 | 9 | 9 | 90 |

* Subcutaneous, single dose; mice used.
† Inoculation into allantoic cavity, single dose.

When a 20-hour-old agar culture of *E. coli* was suspended in water and plated out in nutrient agar containing 5 μ or 25 μ of neomycin per ml, no colonies of *E. coli* developed out of 22 billion cells, after 9 days' incubation at 28° C. Similar concentrations of streptomycin would usually allow the development of a dozen or more bacterial colonies per plate. When pieces of agar were removed from the plates containing neomycin and inoculated into sterile agar plates, only the 5 μ/ml plate gave growth from some of the pieces, but the 10 μ/ml and the 25 μ/ml plates gave no growth at all, thus pointing to the high bactericidal properties of neomycin. When plates containing different concentrations of this antibiotic were streaked with streptomycin-sensitive, -resistant, and -dependent strains of *E. coli* (*9*), the first two were sensitive alike to neomycin, and the last did not make any growth at all. This established further the difference in the biological and chemical nature of neomycin from streptomycin.

Broth or agar cultures containing sufficient neomycin to inhibit the growth of various bacteria were incubated for considerable periods of time. No further bacterial development occurred beyond a certain initial inhibiting concentration, thus pointing on the one hand to the stability of neomycin, as contrasted to that of aureomycin, and on the other hand to the lack of resistance developed among the sensitive bacteria, as contrasted to streptomycin and especially to grisein.

Since neomycin has not yet been obtained in crystalline form, very little can be said of its chemical nature. Preliminary results, however, point to its being distinctly different chemically from streptothricin and from streptomycin. More detailed studies will be published later.

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# TECHNICAL PAPERS

## Electron Micrographic Observations of Tobacco Mosaic Virus in Crude, Undiluted Plant Juice[1]

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Several reports have been published (*2, 3, 4, 5, 6*) of electron microscopic studies of the tobacco mosaic virus in various degrees of impurity. In no study, however, has the infected juice been used in its crude, undiluted form. There has always been some degree of treatment, if only with distilled water, prior to observation in the electron microscope. The published micrographs of the impure material are qualitatively similar to those obtained from freshly purified material.

We have recently obtained electron micrographs of a wild strain of tobacco mosaic[2] grown in White Burley tobacco plants. The specimens for the microscopic examination were prepared in this way: A small portion of the tobacco leaf was crushed by a round-end glass rod in a small glass tube. A drop of expressed juice was picked up in a micropipette, and a bubble was blown over the surface of the specimen screen. The bubble was quickly wiped across the screen, and a very thin film of material was left behind. This film dried almost instantly, and the dried specimen was then shadowed with uranium. The total elapsed time between expressing the juice and drying the film was about 15 sec.

A typical micrograph from a 14-day-old infection is shown in Fig. 1. As can be readily seen, the virus particles appear in the form of large bundles, or sheaves, whose length is roughly 1 to 3 μ, and whose diameter, or width, varies greatly but might average 150 mμ. The background appears quite coarse, since it consists of all of the nonvolatile plant material. Uninfected plants are found to exhibit none of these bundle-like forms. The size and frequency of the typical forms shown in Fig. 1 increase with the age of the infection, being barely visible at 36 hrs after inoculation.

Fig. 2 illustrates the probable reason why previous work with "crude" juice has not exhibited the sheaves of virus particles. The specimen shown in this micrograph was prepared like that in Fig. 2, up to the shadow-casting, but was then washed with two drops of distilled water and allowed to dry. It was then shadowed with uranium. Even such a light and brief treatment with distilled water is evidently sufficient to bring about extensive disintegration of the bundles and to cause many typical, single particles to become visibly detached from the larger groups.

It is well known from observations with the optical microscope (*1*) that needle-like forms are found to exist *in vivo* in the hair-cells of an infected plant, particularly

FIG. 1. Electron micrograph of expressed, crude juice of White Burley tobacco leaf infected with a wild strain of tobacco mosaic virus. Note the bundle-like forms in which the virus is seen. Magnification is 30,000 ×.

FIG. 2. Electron micrograph of the same infected material as is shown in Fig. 1, except that the dried preparation was washed briefly with distilled water. Note that the virus particles are now partially dispersed in individual rods. Magnification is 30,000 ×.

[1] These results have been orally reported at the recent meeting in Toronto of the Electron Microscope Society of America and at the Autumn Conference on Electron Microscopy in Cambridge, England. The work is part of a program supported by a grant-in-aid from the American Cancer Society, on recommendation of the Committee on Growth of the National Research Council.

[2] The initial virus used for propagation in our laboratory was kindly furnished by Gerald Oster.

when the cell has been slightly injured or treated with mild acid. It is entirely possible that what is seen in detail in the electron micrographs is identical with the needle-forms recognized in the optical microscope.

The interesting question in connection with these preliminary results is whether the groups of virus particles exist as such in the intact plant cells, or whether they form subsequent to the crushing of the cells. If the latter is true, they can be considered aggregates of the individual virus particles. But if further work should show that they exist in the intact cells, then the interesting question will arise as to whether or not the virus bundles represent the geometrical growth pattern of the virus.

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## Infrared Spectra and the Amide Linkage in a Native Globular Protein


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The presence of absorption characteristic of amides in the 6-μ region of the spectrum has been observed in proteins by several investigators (*1*, *6*, *8*) and has frequently been cited as evidence against the "cyclol 6" structure postulated by Wrinch (*9*) for native protein molecules. The results reported, however, have been for insoluble, fibrous proteins or for samples in the form of a dried film. In view of the pronounced sensitivity of proteins to drying, as well as to deposition in a film, it has always been possible to cast doubt on this evidence for the amide linkage, at least insofar as native globular proteins are concerned, on the ground that the molecule had been denatured during the experiment.

A protein which is relatively stable to the drying process is serum albumin. It has also been observed recently that native serum albumin produces a modification in the visible spectra of many compounds (*3*) and that this ability is lost on denaturation (*5*). It has seemed appropriate, therefore, to examine the infrared absorption of a dried film of crystallized bovine serum albumin and then to test the sample for possible denaturation by seeing whether it is still capable of causing characteristic spectrophotometric shifts.

The infrared absorption spectrum of bovine serum albumin in the 6-μ region is illustrated in Fig. 1, together with the synthetic, water-soluble, polypeptide, polylysine

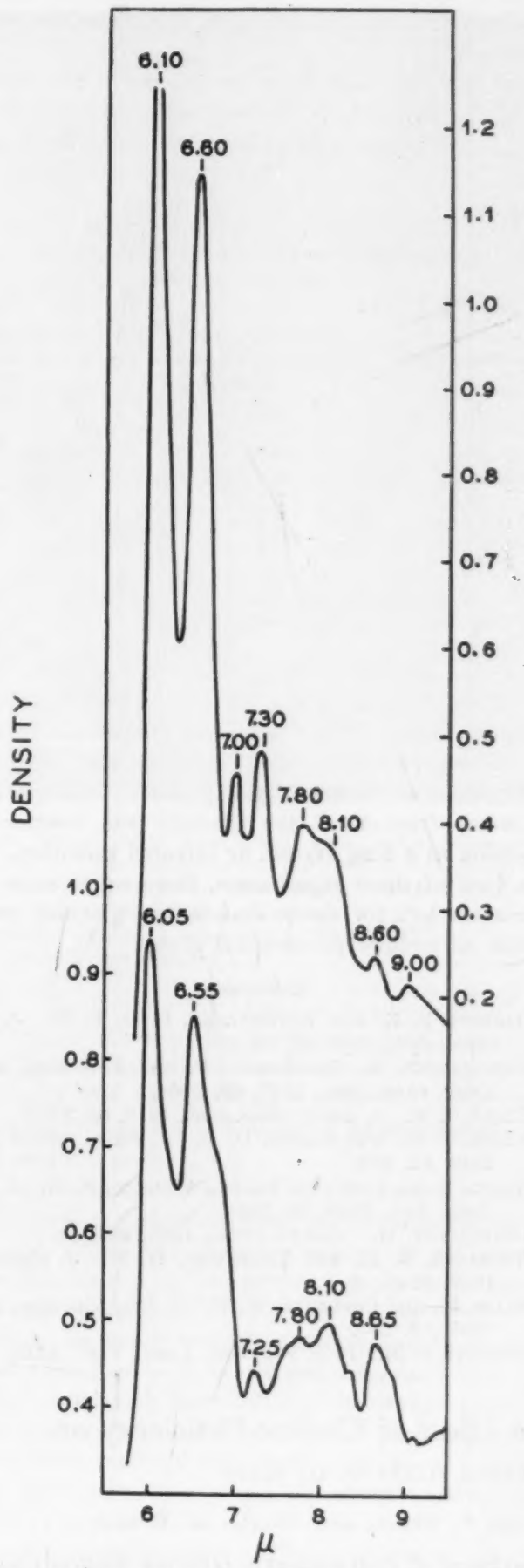


FIG. 1. Infrared absorption spectra of films of bovine serum albumin (top) and polylysine (bottom).

(*2*)[1] for comparison. The C=O peaks at 6.05 and 6.10 μ are essentially identical in position and characteristic of the simple amides in the solid state (*4*, *7*). Thus there can be no doubt that amide carbonyl groups are present

[1] We are very much indebted to E. Katchalski for his generous gift of a substantial sample of polylysine hydroiodide.

in the film of bovine albumin used to obtain the spectrum illustrated.

That this same sample of bovine serum albumin was still in the native form is demonstrated by the data in Table 1. The protein film, dissolved in phosphate buffer

TABLE 1

TEST OF NATIVE CHARACTER OF DRIED PROTEIN FILM BY EXAMINATION OF ITS EFFECT ON SPECTRUM OF METHYL ORANGE

| Wave length (A) | Depression of optical density by protein | |
|---|---|---|
| | From dried film* | Untreated† |
| 4,600 | 0.063 | 0.099 |
| 4,700 | 0.097 | 0.138 |
| 4,800 | 0.117 | 0.165 |
| 4,900 | 0.134 | 0.175 |
| 5,000 | 0.123 | 0.167 |

* Contained 8 mg of protein.
† Contained 12 mg of protein.

at pH 7.6, depressed the optical absorption of methyl orange, as is characteristic of native albumin. Furthermore, the degree of depression, in comparison with that obtained with the control, untreated sample, was roughly in proportion to the quantity of protein. Clearly then, no significant fraction of the albumin was denatured by deposition as a film, drying, or infrared radiation.

In view of these experiments, there would seem to be little room left for doubt that native, globular proteins contain an amide-type carbonyl group.

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## The Effect of Choline-Deficiency on Uterine Activity of Rats


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Peet and Sampson (*7*) reported recently that two post-partum albino rats, one nursing young and one non-lactating, maintained for 39 days on a choline-deficient diet, were found at autopsy to lack completely contractility of the uterus and to have partial atrophy of the uterus and ovaries. Two controls, both nursing young, which received respectively 30 mg and 200 mg of choline every second day, showed normal uterine activity. A yellow, oily condition of the hair on the rats receiving the choline supplements was also noted.

The work reported here was undertaken on a larger group of animals to confirm these observations if possible.

Nine Carworth Farms adult virgin female rats of the Wistar strain were used unmated and nine similar rats were mated. Two of the mated rats died during pregnancy and seven delivered litters, six of these nursing from 7 to 10 young apiece. All virgin rats were placed on the experimental diets at the same time, and each mother was given the experimental diet following delivery of her litter. The diets were distributed among the animals as follows: 3 virgin females on Purina Dog Chow; 3 virgin females, 3 nursing females, and 1 post-partum nonlactating female on the choline-deficient diet; and 3 virgin females and 3 nursing females on the choline-deficient diet plus a choline supplement.

TABLE 1

EFFECTS OF CHOLINE-DEFICIENCY AND LACTATION ON BODY WEIGHT AND ON OVARIAN AND UTERINE WEIGHTS

| Rats & diet | No. in group | Average body weight change | Average ovarian weight | Average uterine weight |
|---|---|---|---|---|
| *Nonlactating virgin rats* | | | | |
| Chow controls .... | 3 | +38 gm | 68 mg | 476 mg |
| Diet controls ..... | 3 | +37 gm | 66 mg | 445 mg |
| Choline-deficient .. | 3 | +39 gm | 59 mg | 421 mg |
| *Nonlactating postpartum rat* | | | | |
| Choline-deficient .. | 1* | +56 gm | 64 mg | 438 mg |
| *Nursing rats* | | | | |
| Diet controls ..... | 3* | −29 gm | 74 mg | 244 mg |
| Choline-deficient .. | 3* | −40 gm | 69 mg | 291 mg |

* One animal from each group was removed from the yeast-casein supplement during the last 14 days of the experiment.

The choline-deficient diet developed by Hegsted and associates (*6*) was used in this work as it was in the work by Peet and Sampson.

The 30 mg choline supplement given every second day was chosen for all control animals in preference to the 200 mg since that amount more nearly approximates physiological levels of the substance (*1, 2, 4, 8*). The additional supplement (*7*) of 1 gm dried brewer's yeast and 5 gm extracted casein was provided every second day to all rats on the basal diet except 3, which were deprived of it completely for the last 14 days of the experimental period in an effort to intensify the choline-deficiency.

Vaginal smears were taken daily and rats were weighed at weekly intervals. All rats were killed by cerebral concussion on the 39th day with the exception of the animals on the Dog Chow diet, which were killed at later intervals when control uteri were desired. At the time of experimentation the ovaries and uteri were rapidly excised, cleaned of excess fat, and weighed.

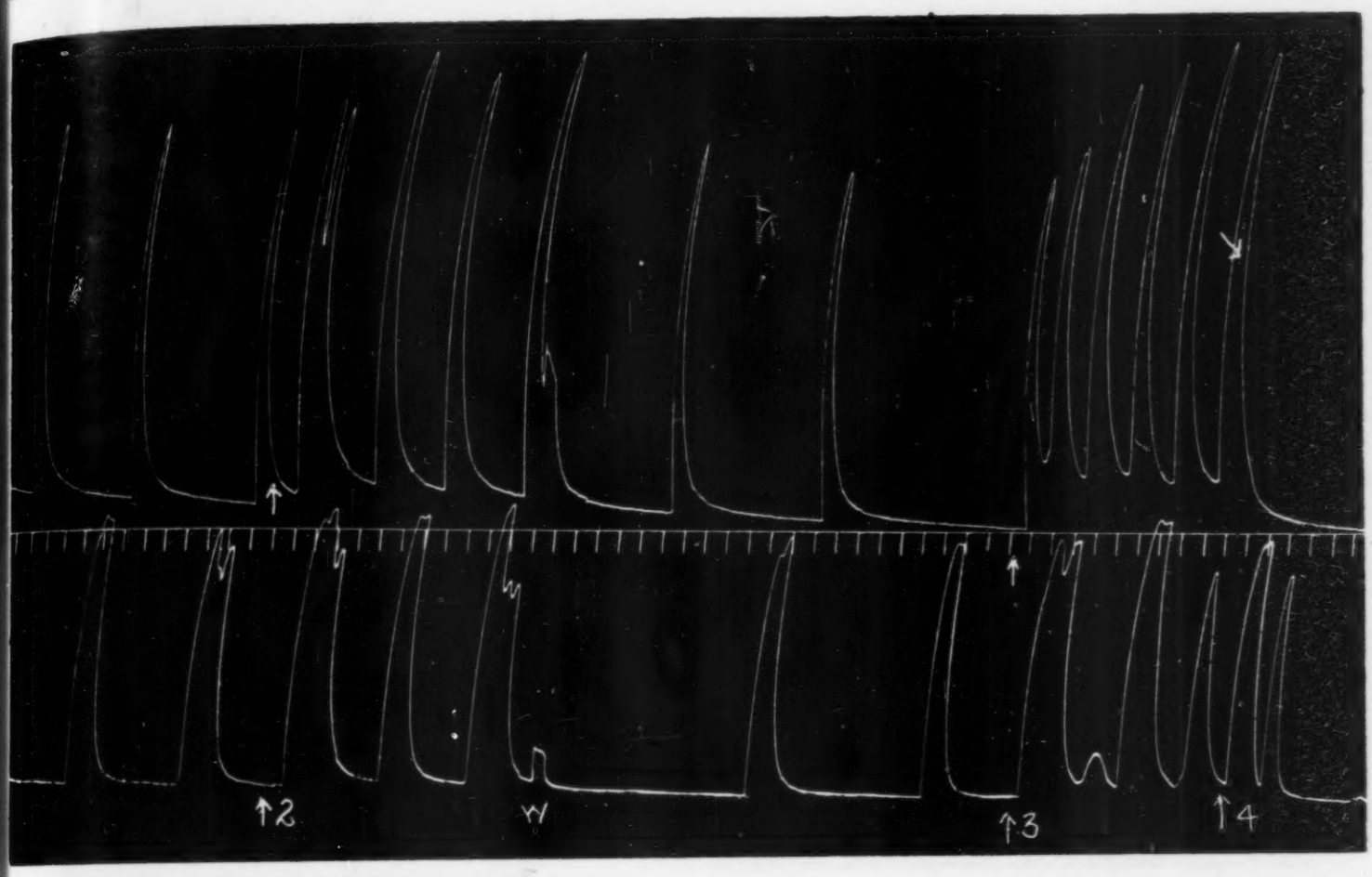


FIG. 1. Top curve shows the spontaneous contractions of the uterus taken from a rat on a choline-deficient diet (animal was deprived of yeast supplement during the last 14 days) and the bottom curve, the activity of a similar segment of uterus taken from a normal rat. Middle record is the time in intervals of 20 seconds. At the interval marked 2, acetylcholine chloride was added to the bath, making a 1 : 25,000,000 solution. At W, the acetylcholine-Locke's solution was replaced by fresh Locke's solution. At 3, posterior pituitary solution (1/250 U.S.P. unit) was added to the 100-cc bath. At 4, epinephrine was added to make a 1 : 2,000,000 dilution.

Segments of one horn of the uterus taken from each of two animals were placed in aerated Locke's solution at 38° C ± 0.2° for recording the contractions on a smoked drum surface. The method used was the same as that previously described by one of us (*5*). Generally, segments of one horn from an experimental animal and of one horn from a control animal were studied simultaneously. All preparations were tested for their response to posterior pituitary solution (1/5th to 1/1,000th unit in 100 cc bath), epinephrine solution (1:2,000,000) and acetylcholine (1: 25,000,000 to 1: 200,000,000).

During the course of the experiment, the fur of all nursing mothers and their litters became oily, apparently about the time the young started to get into the food containers in the cages, while at no time did oiliness of the fur appear in any nonlactating rat.

Table 1 reveals no essential differences in either the nursing or the nonlactating groups between the choline-deficient animals and their controls. There are, however, differences between the nursing animals considered as a group and the nonlactating rats as a group, indicative of an inadequacy of the diet for lactation, despite the addition of choline. Every nursing rat lost weight during the 39-day experimental period while every nonlactating rat gained weight. The uteri of the 6 nursing mothers varied in weight from 168 to 427 gm with an average of 268 gm, while the uteri of 10 nonlactating rats ranged from 318 to 560 gm with an average of 446 gm. The two nursing rats, one choline-deficient and one control, deprived of the yeast and casein supplement for the last 14 days of the experiment showed a marked loss in body and uterine weight, while the non-nursing postpartum choline-deficient rat on a similar regimen appeared entirely normal.

The vaginal smears showed regular four-day estrous cycles in both experimental and control nonlactating females, and continuous diestrous smears in all nursing rats, broken by one or two estrous periods near the end of the experiment.

All uteri, on being excised and placed in Locke's solution at room temperature, contracted spontaneously and remained contracted until placed in warm (38° C) aerated Locke's solution. Immediate spontaneous contractions were recorded from every uterus, with no significant differences in rhythmicity or force of the contractions (Fig. 1).

The rate of contraction and general tonus were increased by both acetylcholine and posterior pituitary solution, while epinephrine caused a decrease in the general tonus and usually cessation of the spontaneous activity in all uteri (Fig. 1). These effects were readily reversed by replacing the drug-Locke's solution by a fresh Locke's solution.

The animals in these experiments represent a rather wide range of choline ingestion from adequacy downward. Although the nine controls received 30 mg of

choline every second day in their diet, the nursing mothers were deprived of a considerable and variable portion of this supplement during the last three weeks of the diet on account of the inroads of their young at the food containers. The experimental animals were obtaining small amounts of choline from the yeast supplement (*3*). The particular product used here provided 3.6 mg of choline every second. The two experimental animals, one nursing and one nonlactating, which were deprived of the yeast and casein supplement for the last two weeks of the diet, probably achieved a true choline-deficiency, but achieved as well a deficiency in protein, biotin, para-aminobenzoic acid, inositol and perhaps other B-complex factors. However, in this widely varied series of 16 rats, spontaneous contractility and normal reactivity to hormonal and humoral agents were observed in all uteri, irrespective of their source.

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## A New Method of Inoculating the Maydeae with Smut Fungi


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Corn seeds soaked in sporidial suspensions of three smuts of the Maydeae showed smut galls soon after germination. This proved to be a new method of infecting corn seedlings with the corn smut organism. The infestation technique employed was the same as that described earlier by the author (*1*) in obtaining seed and seedling infection of the grasses and cereals with *Xanthomonas translucens*. This discovery added another method of inoculating corn with species of Ustilago. The well known method of promoting infection with *Ustilago zeae* is to bring the sporidia or chlamydospores in contact with the growing point down in the spiral whorl or to inject the spores hypodermically into the tissues of the host. Seed infestation with sporidial suspensions in water merely constituted a surface contamination of the seed under favorable conditions for the growth and development of the parasite and the host. Apparently the fungus gained entrance into the host through the germinating seed and eventually reached the crown of the young developing seedling. Infection occurred only when the seeds were infested with sporidia and not with

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TABLE 1

The Response of Sweet and Dent Corn Infested in Sporidial Suspensions of Three Species of Smut Fungi

| Smut species | No. seeds infested | No. plants emerged | No. plants infected |
|---|---|---|---|
| | | *Sweet corn** | |
| *Ustilago zeae*† | 100 | 82 | 32 |
| *Ustilago dieteliana*‡ on Tripsacum | 260 | 210 | 81 |
| *Sphacelotheca reiliana* from teosinte | 360 | 322 | 0 |
| | | *Dent corn*§ | |
| *Ustilago zeae*† | 100 | 97 | 2 |
| *Ustilago dieteliana*‡ on Tripsacum | 420 | 400 | 29 |
| *Sphacelotheca reiliana* from teosinte | 360 | 348 | 0 |
| | | *Teosinte*‖ | |
| *Ustilago zeae*† | 40 | 25 | 0 |
| *Ustilago dieteliana*‡ on Tripsacum | 20 | 13 | 2 |
| *Sphacelotheca reiliana* from teosinte | 40 | 19 | 0 |

* Sweet corn variety Golden Bantam.

† *Ustilago zea* was collected near Ames, Iowa, and grown in single and mixed sporidial cultures for the infection trials.

‡ *Ustilago dieteliana* was collected by I. E. Melhus in 1945 near Mexico City, Mexico, on *Tripsacum latifolium*. Mixed sporidial cultures were used in the infection trials.

§ Dent corn commercial hybrid U. S. 13.

‖ Teosinte obtained from field near Chalco, Mexico.

chlamydospores. Some seedlings were killed immediately after emergence. Others developed large swellings or galls, and still others were not attacked. This method of infection was not limited to *Ustilago zeae*. Infection was

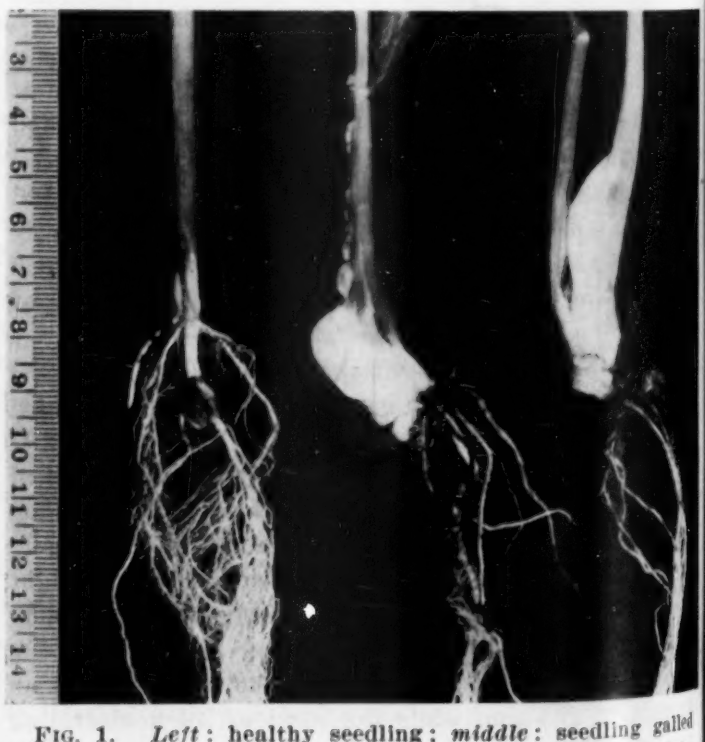

Fig. 1. *Left*: healthy seedling; *middle*: seedling galled by Tripsacum isolate; *right*: seedling galled by corn isolate.

obtained with the sporidia of other smuts on corn and teosinte as indicated in Table 1.

The results of the seed infestation trials indicated that sweet corn was more easily infected when soaked in sporidial suspension than either dent corn or teosinte. Also, the response of the Tripsacum smut was like the corn smut organism in that *Ustilago dieteliana* occurring on *Tripsacum latifolium* was pathogenic on corn (Fig. 1).

Upon finding that galled seedlings developed from seeds infested in sporidial suspension, before planting a search was made for infected seedlings in field plots on land that had formerly grown corn. In the spring of 1946 infected seedlings from 4″ to 6″ tall were found. Subsequently a similar search was made in adjoining fields. Again infected seedlings were found. Most of these seedlings were killed before they were 8″ tall.

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## The Semiquinone Radical of Tocopherol

L. Michaelis and S. H. Wollman[1]

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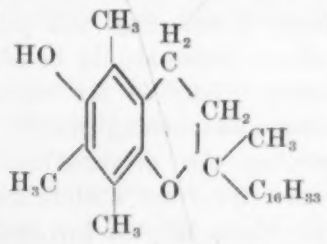
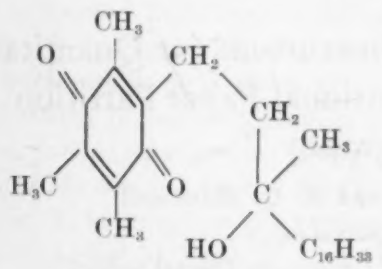
The structure of tocopherol (vitamin E) suggested the idea that its function both as a vitamin and as an antioxidant might be correlated to some reversible oxidation-reduction mechanism. However, this idea is difficult to reconcile with our present knowledge of the oxidation products of tocopherol (I). The first oxidation product



(I) α-Tocopherol

attainable has the structure of a quinone (II) which can be reduced, but the reduction product is not the original tocopherol. The oxidation of tocopherol appears to be irreversible. To quote from a review of this problem (*6*),



(II) The quinone

this fact "militates against the idea that the compound (viz. the quinone) enters into the utilization of the vitamin in the body through an oxidation-reduction process."

The antioxidant property of tocopherol is shared with hydroquinone (benzo-hydroquinone). The structure of tocopherol differs from that of hydroquinone and that of duro-hydroquinone (= tetramethyl hydroquinone) essentially in that it contains only one unsubstituted phenolic hydroxyl group, instead of two. This is why a reversible oxidation to a quinone cannot take place. This fact suggests that the antioxidant effect of hydroquinone may not be due to its reversible oxidation to quinone. However, the structure of tocopherol does not rule out the possibility of its reversible univalent oxidation to a semiquinone radical, which would arise from (I) by the removal of one electron only, in analogy to the semiquinone of duroquinone as described previously from this laboratory (*5*) and to many other semiquinones (*3*, *4*). This process would not involve opening the side-ring, as is necessary for the bivalent oxidation to the quinone and does not imply any irreversibility of the oxidation.

Such a hypothesis is difficult to prove for tocopherol because even if a radical is formed it may never accumulate to any concentration directly observable. The hypothesis would be made more acceptable, however, if one would succeed in making the free radical under certain artificial conditions at such a high concentration that one could easily detect it directly. For this purpose an ingenious method devised by G. N. Lewis (*2*) is helpful:

The substance in question is dissolved in an organic solvent which at the temperature of liquid air will form a homogeneous glass instead of crystallizing. It is irradiated, at the low temperature, in a Dewar flask through a quartz window, with ultraviolet light from a mercury lamp. Radiation may have two effects: It raises the energy level of some electron in the molecule, and when the excited state spontaneously returns to the ground state, there may be fluorescence, or phosphorescence, according to conditions; or irradiation may knock out an electron entirely and produce a free radical. Under ordinary conditions, the concentration of such a free radical would be restricted by the fact that thermodynamical equilibrium is established, for instance, due to dismutation of the radical. In most cases, the equilibrium is very much in disfavor of the free radical. At the low temperature in the rigid solvent, however, molecular collisions necessary to establish equilibrium are inhibited, and the free radical can accumulate to a concentration far above its thermodynamically permissible equilibrium concentration. The radical can be recognized by its characteristic color. On warming the system to a temperature somewhat above that of liquid air, the radical gets into the equilibrium concentration, which is extremely small. The color of the radical fades out on raising the temperature but can be preserved almost indefinitely as long as the vessel is kept at the temperature of liquid air.

With this method it has now been shown that tocopherol, dissolved in a mixture of alcohol, ether, and pentane, frozen and irradiated, develops with great ease and rapidity an orange-red color, showing characteristic absorption bands, which is stable at the temperature of liquid air even after stopping the radiation, but fades out on melting the frozen solution.

The color of the semiquinone of hydroquinone, prepared in the same way, is light yellow. This yellow substance is not quinone since it fades out on warming, but

[1] Special research fellow of the National Cancer Institute.

is the semiquinone radical. The monomethyl-ether of hydroquinone behaves as hydroquinone, which shows that one unsubstituted phenolic hydroxyl group is sufficient for the reaction and corroborates the assumption that the yellow color is not quinone.

There can be no doubt that this colored substance is a free radical existing in a concentration far above the thermodynamically permissible concentration. This experiment shows at least that the free radical of tocopherol is not just a product of paper chemistry but does exist. At ordinary temperatures, it will never accumulate to a directly recognizable extent. On the other hand, its very instability makes it a highly reactive substance.

Although the univalent oxidation may involve a large free energy, the process might be greatly facilitated if tocopherol were a prosthetic group of an enzyme and the process took place intramolecularly within the enzyme-substrate complex. Such an assumption would also fit the fact that the biological activity is restricted to a molecule of specific structure and is not exhibited generally by any mono-substituted hydroquinone.

It is not claimed that the problem as to the mechanism of the action of tocopherol either as a vitamin or as an antioxidant is entirely solved herewith. But the fact that the finding of the semiquinone places tocopherol in the class of the reversible oxidation-reduction systems establishes the analogy to various other vitamins which act as coenzymes or prosthetic groups of enzymes concerned with specific dehydrogenations, namely, riboflavin, nicotinic acid, and thiamine.

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## Technique for Making Spreads of Omentum from Small Animals[1]


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Possibly one outcome of the work of Knisely's group (*2*) on sludged blood will be a re-awakened interest in the potential usefulness of the omentum in studies of the vascular system. Because the omentum is very thin, sectioning is unnecessary for microscopic studies, and so the observer can secure an unmutilated and complete survey of the vascular bed over a relatively wide field (*1*).

Smooth and sizable spreads of this tissue, however, are not easily obtained because as soon as the anchorage of the omentum to the intestine is destroyed, the mesentery tends to contract and wrinkle badly. The following technique routinely produces smooth spreads of omentum from mice (half grown to adults in size).

The tissue should be taken as soon after death as possible. The use of a duoloupe or dissecting microscope is advantageous. The intestine should be spread out so that a loop bounds a fan-shaped portion of the omentum, avoiding the region of the pancreas if very thin preparations are desired. A slip of cigarette paper (cut crosswise in about thirds) is placed on a gloved fingertip and inserted under the chosen portion of the omentum, retaining the fingertip beneath the omentum until the preparation is placed on a slide. The membrane is spread smoothly against the paper. Unless too much time has elapsed since the death of the animal, the albuminous substances in the tissue will affix it to the paper. The intestinal loop and enclosed "fan" are severed by fine scissors. The cut loop is carefully trimmed away from the enclosed membranous "fan" by cutting through the membrane and the supporting paper. The chosen portion of the paper-supported omentum is left balanced on the fingertip and then gently but firmly pressed with the tissue side down against the slide. Using scissors' tips or needles the spread is smoothed before it is covered with a few drops of fixing solution (we used Schaudinn's). After a delay of a second or so, the spread is blotted dry quickly by pressing with several layers of filter paper.

The next step requires speed, care, and skill. With a fine-pointed forceps for lifting the paper and a teasing needle or scissors' tip to hold the tissue against the slide, the cigarette paper is separated and peeled away. The slide with the affixed tissue should be placed in a jar of fixative immediately. Any of the usual techniques for fixing, dehydrating, and staining paraffin sections may be used for completing the preparation. If, as rarely happens, a spread slips from a slide during these later manipulations, the tissue may be salvaged by handling in the customary manner for "loose" celloidin sections.

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## Scanning Instrument for Quantitative One-Dimensional Paper Partition Chromatography


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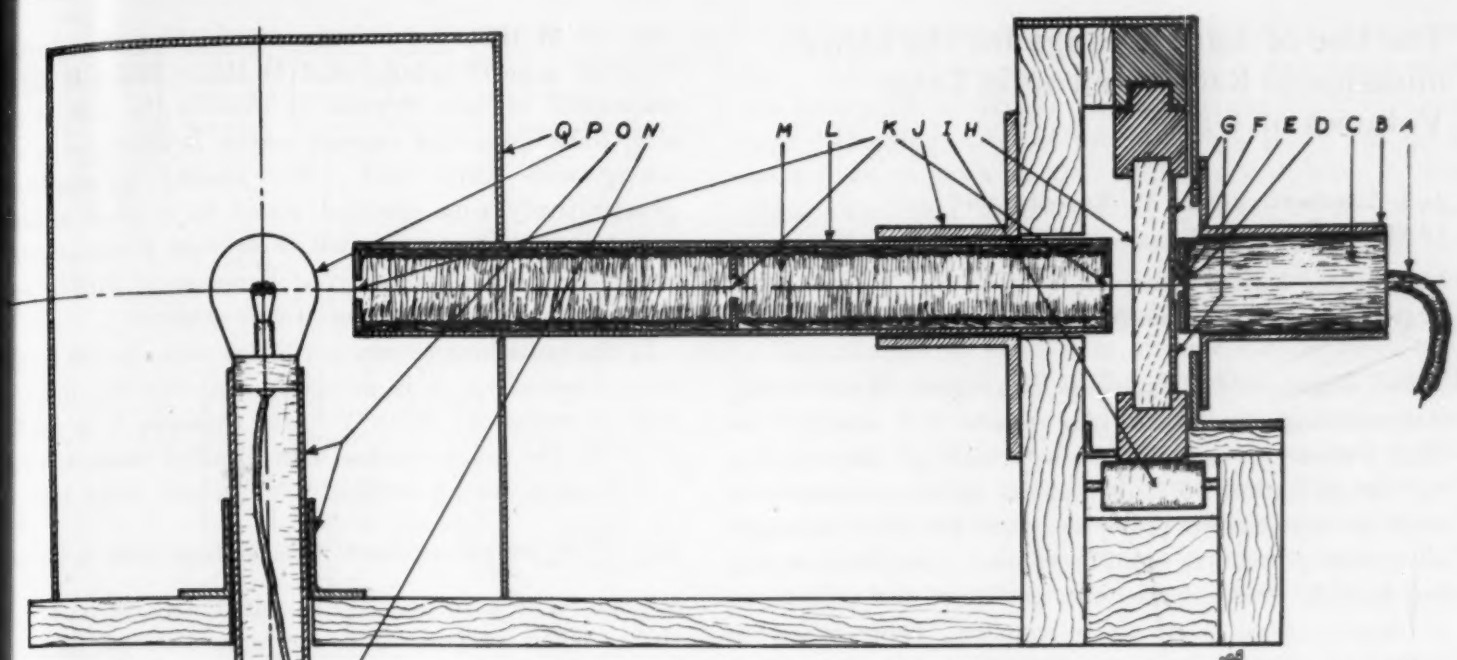
The method of Consden, Gordon and Martin (*3*) for separation and identification of amino acids by paper partition chromatography has found wide application and has been extended to other classes of compounds. Various methods for rendering the method quantitative have been described (*4, 5, 6, 7, 8, 9, 10, 11*). In this laboratory the method developed by Bull and coworkers (*1*)

[1] Supported in part by grants from the U. S. Public Health Service and the Office of Naval Research.




A. Lead to Model 500 Electronic Photometer
B. Metal Housing for Photoelectric Cell
C. Model D Photoelectric Cell
D. Wratten Filter G15 (Eastman)
E. Window in Photoelectric Cell
F. Filter Paper Strip
G. Brass Masking Strip
H. Glass Slide
I. Supporting Roller
J. Metal Housing for Collimating Tube
K. Circular Apertures
L. Collimating Tube
M. Black Felt Lining
N. Leads to 6 Volt Transformer
O. Lamp Support
P. Lamp 32 Candle Power
Q. Metal Housing

FIG. 1.

n quantitative estimation of amino acids by one-dimenional paper partition chromatography is being used. his method, also developed by Block (*1*), depends upon he measurement of transmission of light through the lter paper strip containing purple spots developed by he reaction of ninhydrin on the individual amino acids n the strip.

The scanning instrument used in this laboratory for easuring transmission through the paper strips is shown n Fig. 1. The photoelectric cell, C, is connected by lead to a photometer (Photovolt Corp.). The filter, D, is ounted in E, the cell window. The photoelectric cell ts snugly in the brass housing, B; the optimal distance etween the paper strip and the cell can be adjusted by iding the latter forward or backward in the housing. he housing, B, is held in place by four set screws (not hown) and is adjustable both horizontally and vertically o facilitate positioning the cell window, E, exactly in ine with the collimated light beam passing through the aper strip, F. The glass slide, H, mounted in metal trips, moves horizontally on a series of supporting roll-rs, I. Brass strips, G, mask the entire surface of the lass slide except a narrow portion along the middle of he face of the slide covered by the filter paper strip, F. he light source, P, mounted on the vertically adjustable upport, O, is connected by leads, N, to a 110 × 6-v trans-ormer (not shown). The cylindrical housing, Q, encloses he light source. The cylindrical brass collimating tube, , fits in the brass housing, J, and can be moved forward r backward for optimal adjustment. The three circular pertures, K, in the collimating tube consist of steel ashers fitted into the tube. The inside of the colli-ating tube is lined with nonreflecting black felt, M. scale, 70 cm in length, graduated from 0 to 100, is inscribed on the instrument frame adjacent to the top of the slide, and an indicator line on the slide frame is used to read the scale. In practice all strips are run in the vapor chamber to a total distance of 70 cm (*i.e.*, a distance equal to the length of the scale on the scanning instrument). The color is developed on the strips (*2*) which are then fastened in the proper position on the glass slide between the brass masking strips. Transmission readings are taken at intervals of ½ scale units (1 scale unit = 0.7 cm) by moving the slide. The transmission readings from the Photovolt meter are plotted directly upon semilog paper as a function of distance in scale units, and the curves treated as described by Bull (*2*). Using this method the $R_f$ value (*3*) can be read directly from the plot.

Accuracy of determinations of amino acids using color development with ninhydrin by Bull's method (*10*) vary for us from 5 to 15% depending upon the amino acid.

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# The Use of Ion Exchange for the Determination of Radioelements in Large Volumes of Urine[1]

**Jack Schubert, Edwin R. Russell,[2] and Lawrence B. Farabee[3]**

***Medical and Biology Divisions,***
***Argonne National Laboratory, Chicago***

The efficacy of ion exchange as a means of extracting, concentrating, and purifying cations and anions from large volumes of solutions is well known. Inasmuch as the determination of radioelements or trace elements in urine involves similar problems it was felt that analogous adsorption procedures could be applied. Numerous studies had already demonstrated the ability of ion exchangers to extract ions, particularly calcium, from biological fluids (*1*). Our objective was to extract quantitatively a trace element from a liter or more of urine and concentrate it in a few ml of a solution completely free from the large concentration of salts which usually interferes with spectrographic and chemical methods of analysis.

In connection with various metabolic studies it was desirable to determine quantitatively the concentrations of fission products in urine, especially with regard to relating the urinary levels of radioelements to body content. Since the rare earths constitute an important fraction of the fission products, the determination of these elements in urine by using a cation exchanger was attempted. Carrier-free radioyttrium, $Y^{91}$, was chosen as a tracer element for these studies. An extensive investigation of optimum conditions was made, but only the most recent workable flow sheet is presented.

The principles involved in the adsorption and desorption of a cation from an organic cation exchanger are discussed elsewhere (*2, 3*). Essentially the cation exchange reactions follow the law of mass action. A given reaction, then, can be made to proceed in any direction by altering the relative concentration of the reactants and products. In this adsorption process for extracting and subsequently eluting yttrium from urine, the principal reaction for both the adsorption and desorption steps is:

$$Y^{+++} + 3HR \underset{\text{Desorption}}{\overset{\text{Adsorption}}{\rightleftharpoons}} YR_3 + 3H^+,$$

in which the symbol *R* represents the nondiffusible anionic part of the cation exchanger.

The adsorption of the yttrium is made from urine, 0.1 M in HCl, while the desorption is carried out with 7 M HCl. Briefly, the adsorption process consists of passing a liter of acidified urine containing tracer yttrium through an adsorption column of a cation exchange resin, removing the extraneous calcium and other cations with 0.4 M HCl, and subsequently eluting the adsorbed $Y^{+3}$ with a small volume of 7 M HCl. The 7 M HCl is evaporated to near dryness to remove the bulk of the acid, while remaining organic matter is destroyed by wet ashing with nitric acid. The residue is transferred quantitatively with distilled water to a 10-ml capacity porcelain capsule, evaporated to dryness under an infrared bulb, and the radioactivity is measured directly with an end mica-window Geiger-Müller counter.

If the radiations from a particular radioelement are not very penetrating, it is necessary that the last traces of salts be removed. With $Y^{+3}$, for example, it is possible to dilute the ashed residue with distilled water and pass it through a second column of adsorbent which contains only $\frac{1}{17}$ by weight as much resin as is present in the first column. The adsorbed $Y^{+3}$ is eluted with 60 ml of 7 M HCl. When evaporated to dryness, mounted on a flat platinum plate, and flamed, this solution leaves, in most cases, no perceptible residue.

The advantages claimed for the method are: (1) no preparation of the urine other than acidification is necessary; (2) no close control of pH is required; (3) the operation may be interrupted at any stage desired; (4) one technician can run several samples simultaneously; and (5) the equipment and adsorbent, once set up, may be used repeatedly.

Amberlite IR-1 resin[4] is employed in the first column adsorption step while a more insoluble resin, Amberlite IR-100H, is used in the second step because it helps to minimize the amount of solid matter associated with the yttrium. The weights of resin are expressed in terms of the air-dried hydrogen form. Now that more insoluble and higher capacity resins, such as Dowex 50[5] (2) and Amberlite IR-120, are available it would be definitely advantageous to substitute them for IR-1 and IR-100H after making allowances for the capacity and kinetic characteristics of these new resins (*2, 4*).

The glass columns are set up in the usual manner. Glass wool serves as a satisfactory support for the resin bed. A straight glass stopcock is used to regulate the flow rates. A 2-l leveling bottle serves as a storage tank for liquids percolating through Column I while a 500-ml leveling bottle suffices for Column II. About 2′ of head is found to provide satisfactory flow rates. The urine was "spiked" with carrier-free $Y^{91}$ and then acidified as specified in the flow sheet.

The accompanying flow sheet (Fig. 1) provides all the details of the operations necessary for the extraction and concentration of $Y^{91}$ from liter volumes of urine. Larger volumes of urine can be handled by a proportionate increase in the mass of resin employed. The resin is ready for a new run at the end of each cycle.

The functions of the different solutions employed are as follows:

(1) The acidification of the urine prevents the precipitation of calcium salts, keeps the yttrium in ionic form

[1] The work reported here is based on studies completed in the autumn of 1945 and is scheduled for publication in Vol. 16A of the Plutonium Project Record.

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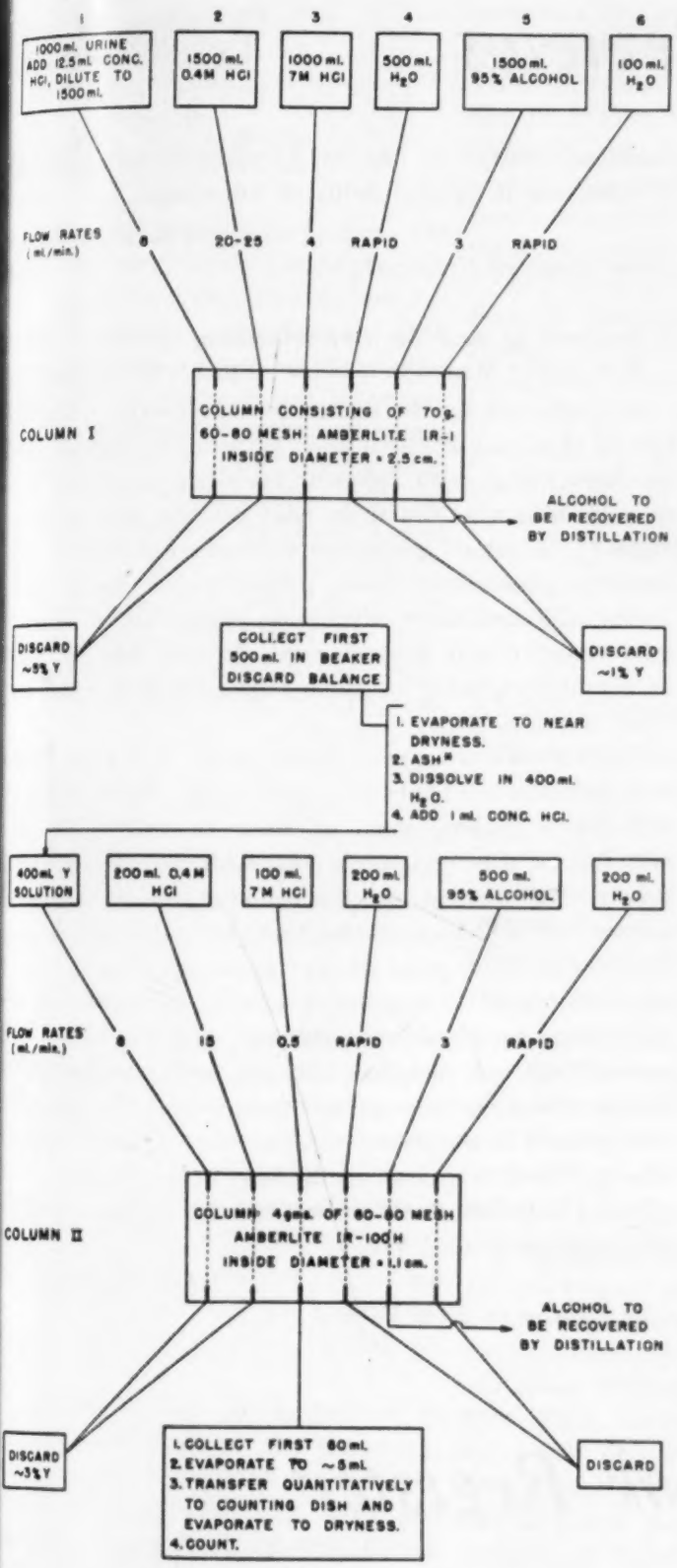
[4] Manufactured by the Resinous Products Chemical Co., Washington Square, Philadelphia, Pennsylvania.

[5] Manufactured by Dow Chemical Co., Midland, Michigan.




*THE SOLUTION, CONTAINED IN A 1 LITER BEAKER, IS EVAPORATED ON A HOT PLATE TO NEAR DRYNESS. THE ORGANIC RESIDUE IS DESTROYED BY THE ADDITION OF ABOUT 20 ml. OF 11 N $HNO_3$ AND EVAPORATED TO DRYNESS. THIS TREATMENT IS REPEATED AT LEAST TWO MORE TIMES. FINALLY TO THE ASHED MATERIAL IS ADDED 10 ml. OF 11 N $HNO_3$ AND THE MIXTURE IS EVAPORATED TO NEAR DRYNESS.

FIG. 1. Flow sheet for the determination of radio-yttrium, $Y^{91}$, in human urine by cation exchange.

and breaks up any unadsorbable complexes that yttrium may form with urinary constituents.

(2) The 0.4 N HCl removes some adsorbed organic matter as well as calcium and other monovalent and divalent cations.

(3) The 7M HCl serves to elute the yttrium quantitatively in a small volume.

(4) The 95% alcohol removes remaining organic matter. This step was introduced when it was found that the adsorption of yttrium from liter quantities of urine kept decreasing, *i.e.*, the break-through point was quite erratic and as much as 35% of the yttrium lost. It was presupposed that alcohol-soluble organic matter coated the resin particles so that the adsorption of yttrium was hindered. The alcohol wash was found to prevent the premature break-through of yttrium.

The adsorption method as it now stands gives consistent recoveries of $Y^{91}$ of $90 \pm 5\%$ when only Column I is utilized, and an over-all recovery of $85 \pm 5\%$ if the process includes Column II. It is possible to improve this recovery by closer control of variables. The effect of yttrium concentration on its adsorption by the resin may be calculated (*4*). In the present process it appears that about $10^{-4}$ M of yttrium can be extracted with no loss in efficiency.

Because the concentrations of substances such as albumin and urea which normally appear in urine are quite variable, it was desirable to ascertain their effects on yttrium adsorption.

*Albumin*: The presence of albumin in urine as high as 2 gm/l had no observable effect.

*Urea*: In the range of concentration studied, 0–4.0 gm of urea/100 ml of urine had no effect on yttrium adsorption.

*Acids*: The equilibrium adsorption of yttrium by IR-1 from urine acidified with each of the following acids was studied: acetic, sulfuric, hydrochloric, and nitric in concentrations ranging from 0.1 N to 1.0 N. The maximum adsorption (which was about the same for the various acids) was obtained when the solutions were 0.1–0.2 N. Higher concentrations of acids caused a marked decrease in yttrium adsorption.

*Sodium chloride*: Concentrations of NaCl up to 1.2% did not reduce the adsorption of yttrium.

In general, the scheme given here will be found suitable for the extraction and concentration of other cations with a valence of +3 or greater. The processing of divalent cations such as $Ba^{++}$ and $Ra^{++}$ would involve a closer attention to the concentration and volume of the HCl-eluting solution in order to remove selectively urinary calcium. Chromatographic complex elution with citrate solutions can, of course, be utilized for the separation of calcium from other divalent cations (*4*). For metals which easily form complex anions, such as chromium, gold, and molybdenum, for example, an anion exchange system (*5*) may be found useful.

## References

1. APPELZWEIG, N. *Ann. N. Y. Acad. Sci.*, 1948, **49**, Art. 2, 295.
2. BAUMAN, W. C., and EICHHORN, J. *J. Amer. chem. Soc.*, 1947, **69**, 2830.
3. BOYD, G. E., SCHUBERT, J., and ADAMSON, A. W. *J. Amer. chem. Soc.*, 1947, **69**, 2818.
4. SCHUBERT, J. "Applications of Ion Exchange to the Separation of Inorganic Cations." Chapter in book, edited by F. C. Nachod, entitled: *Ion exchange—theory and practice*. New York: Academic Press. 1949.
5. SUSSMAN, S., NACHOD, F. C., and WOOD, W. *Ind. eng. Chem.*, 1945, **37**, 618.

# Book Reviews

***Advances in food research.*** (Vol. I.) E. M. Mrak and George F. Stewart. (Eds.) New York. Academic Press, 1948. Pp. xv + 459. (Illustrated.) $7.50.

This book contains reviews on the following subjects: the physiology and chemistry of rigor mortis, with special reference to the aging of beef; factors influencing the vitamin content of canned foods; the physiological basis of voluntary food intake (appetite); biochemical factors influencing the shelf life of dried whole eggs and means for their control; factors affecting the palatability of poultry with emphasis on histological post-mortem changes; deterioration of processed potatoes; the influence of climate and fertilizer practices upon the vitamin and mineral content of vegetables; nonenzymatic browning in fruit products; microbial inhibition by food preservatives; and high-polymer pectins and their deesterification.

Each of the reviews is accompanied by a suitable bibliography to the sources of information on which it is based. Every contributor is an experienced investigator in his segment of food research.

Researches on foods have been accelerated by the great advances in our knowledge of biochemistry and the biological sciences. Original communications are scattered in journals devoted to biochemistry, bacteriology, mycology, and nutrition. Technologists, researchers, and teachers find it almost impossible to examine the original literature which bears upon their interests. In various fields of knowledge the annual volume devoted to thorough reviews and criticism has done much to assist those interested in keeping informed about research progress. The references help them to find readily what they most need to examine at first hand. The present volume is an excellent addition to the list of special volumes devoted to advances in special fields of knowledge.

E. V. McCollum

*Johns Hopkins University*

***A textbook of heat for upperclassmen.*** LeRoy D. Weld. New York: Macmillan, 1948. Pp. x + 437. $5.00.

As explained by the author in the preface, "The content of this book is based upon material which the author has been using, with frequent revisions, as lecture notes in a junior-senior course in heat for the past thirty-five years." A lot of ground is covered: calorimetry, dilatometry, phase transitions, properties of gases, kinetic theory of gases, thermodynamics, properties of mixtures, heat transfer, and thermometry. In less than 400 pages of actual text, many of these topics could be given only brief mention.

The author has an easy, fluent style. His explanations and examples are lively and interesting. There are many well-drawn graphs, some of them to scale. The best material in this reviewer's judgment is that on kinetic theory—the poorest on the foundations of thermodynamics. It is to be regretted that the author has retained the old-fashioned point of view with regard to the use of the term "heat"—a point of view at variance with current usage by physicists, chemists, and engineers. One cannot help but complain also about the author's symbolism which ignores almost completely the published lists printed in the *American Journal of Physics* and elsewhere. There is no excuse, in these times, for using complicated bold-face Gothic characters for thermal capacity, efficiency, resistance, etc.

Mark W. Zemansky

*City College of New York*

# Scientific Book Register

Barnes, H. F. *Gall midges of economic importance: gall midges of ornamental plants and shrubs.* (Vol. IV.) London: Crosby Lockwood, 1948. Pp. 165. (Illustrated.) 15/–.

Brill, A. A. *Basic principles of psychoanalysis.* Garden City, New York: Doubleday, 1949. Pp. xv + 298. $3.45.

Buros, Oscar Krisen. (Ed.) *The third mental measurements yearbook.* New Brunswick, N. J.: Rutgers Univ. Press, 1949. Pp. xiv + 1047. $12.50.

Chance, Britton, *et al.* (Eds.) *Waveforms.* (Massachusetts Institute of Technology Radiation Laboratory Series.) New York-London: McGraw-Hill, 1949. Pp. xxii + 785. (Illustrated.) $10.00.

Clarke, Hans T., Johnson, John R., and Robinson, Sir Robert. (Eds.) *The chemistry of penicillin: report on a collaborative investigation by American and British chemists under the joint sponsorship of the Office of Scientific Research and Development, Washington, D. C., and the Medical Research Council, London.* (Compiled under the auspices of the National Academy of Sciences, Washington, D. C.) Princeton, N. J.: Princeton Univ. Press, 1949. Pp. x + 1094. (Illustrated.) $36.00.

Zipf, George Kingsley. *Human behavior and the principle of least effort: an introduction to human ecology.* Cambridge, Mass.: Addison-Wesley, 1949. Pp. xi + 573. (Illustrated.) $6.50.

# NEWS and Notes

**National Science Foundation.** On March 18, the Senate passed S. 247, the "National Science Foundation Act of 1949." On the same date, the Public Health, Science, and Commerce Subcommittee of the House Interstate and Foreign Commerce Committee announced that hearings on the National Science Foundation bills will be scheduled for March 31, April 1, and April 4 at 10:00 a.m. As noted in the March 18 issue of *Science*, one of the chief purposes of the hearings is to familiarize new Subcommittee members with the legislation, but Congressman J. Percy Priest has invited all interested organizations and individuals to present their views.

By invitation of Congressman Priest, the Inter-Society Committee for a National Science Foundation will be represented by Dael Wolfle, secretary-treasurer of the Committee; and the AAAS will have Howard A. Meyerhoff, administrative secretary, and Raymond L. Taylor, assistant administrative secretary, present to answer any questions the House Subcommittee may ask. Such support as these three representatives can give to a National Science Foundation Bill will be greatly strengthened by letters from scientists addressed to members of the House Subcommittee (see *Science*, March 18, p. 295).

Members of the Subcommittee on Public Health, Science and Commerce have six bills before them for consideration. H. R. 12, 185, 311, 1845, and 2308 differ only in minor details from H. R. 6007 of the 80th Congress and from the Senate bill S. 247. Sponsors of these bills are: *For H. R. 12*, J. Percy Priest (6th District, Tennessee); *for H. R. 185*, Oren Harris (7th District, Arkansas); *for H. R. 311*, Charles A. Wolverton (1st District, New Jersey); *for H. R. 1845*, James E. Van Zandt (22nd District, Pennsylvania); *for H. R. 2308*, Wilbur D. Mills (2nd District, Arkansas). For all practical purposes these five bills may be considered as a single piece of legislation, which will receive the united support of their respective proponents. The only rival bill is H. R. 359, introduced by Emanuel Celler (15th District, New York). This bill closely follows the pattern set by S. 1850 of the 79th Congress, which, though supported by many of the nation's scientists, was the subject of vigorous controversy.

Only once (1947) has a National Science Foundation bill passed the House. If scientists wish to insure passage of a bill in 1949, it will help if they express themselves to their Congressmen, to the proponents of these six bills, and to the members of Mr. Priest's Subcommittee, not only in support of the Foundation, but also in regard to the kind of Foundation they want.

HOWARD A. MEYERHOFF

## About People

**Clark B. Millikan,** professor of aeronautics, California Institute of Technology, has recently been named director of the Daniel Guggenheim Laboratory of Aeronautics and chairman of the Jet Propulsion Laboratory Board at Caltech.

**Milan V. Novak** has been appointed assistant dean of the Graduate College for the University of Illinois' Chicago Professional Colleges. Dr. Novak will continue to serve as professor of bacteriology and head of the Department.

**J. William Hinton,** a professor of clinical surgery on the New York University medical faculty since 1944, has been appointed professor of surgery at the Post Graduate Medical School, New York University-Bellevue Medical Center. Dr. Hinton will be director of the fourth surgical division at Bellevue and will continue as director of surgery at University Hospital.

**Henry G. Booker,** professor of electrical engineering at Cornell University, has been elected Honorary Fellow of the Geophysical Observatory, University of Alaska. This citation honors him for his work on microwave propagation and electromagnetic wave propagation in the upper atmosphere.

The National Bureau of Standards announces two appointments to the staff: **Charles A Mabey,** former director of research for the Bristol Company, will supervise electronic miniaturization, circuits, and processes as assistant chief of the Engineering Electronics Laboratory; and **Paul Wang,** former dean of the Physics Department, Shantung University, China, will do research in X-rays and nuclear physics, specializing in betatron and high-voltage equipment.

**Douglass Poteat,** former executive vice president of the American Cancer Society, has recently been appointed executive vice president of Memorial Hospital Center for Cancer and Allied Diseases in New York City.

**Edward O. Hulburt,** former superintendent of the Optics Division, Naval Research Laboratory, Washington, D. C., has been made director of research of the Laboratory.

## Grants and Awards

**The Institute of Medicine of Chicago** is offering the Joseph A. Capps prize of $400 for the best paper on a medical study or a problem closely related to medicine. The competition is open to graduates of Chicago medical schools who completed their internship or one year of laboratory work during 1947 or later. Manuscripts must be submitted to the Secretary of the Institute of Medicine of Chicago, 86 East Randolph Street, Chicago 1, not later than *December 31*.

**The Commonwealth Fund** has awarded an advanced medical fellowship to David Glick, associate professor of physiological chemistry, University of Minnesota Medical School, to study developments in histo- and cytochemistry at the Carlsberg Laboratory in Copenhagen and the Kaolinska Institute in Stockholm during the summer.

**Elwood C. Zimmerman,** associate entomologist, Experiment Station, Hawaiian Sugar Planters' Association, and curator of entomology, Bishop Museum, was awarded the 1948 literary prize of $250 by the Friends of the Library of Hawaii for his five-volume work "Insects of Hawaii." This is the first time a scientific treatise has been given the award.

The Institute of Radio Engineers gave its Fellow Award this year to **John N. Dyer,** supervisor of radar and air navigation research and development for Airborne Instruments Laboratory, Mineola, Long Island, New York. Mr. Dyer was honored for his

contributions to radio, including polar expedition communications and wartime radio counter measures.

Sigma Delta Epsilon, national women's scientific fraternity, has awarded its $1,200 predoctoral fellowship for 1949–1950 to **Irene T. Kline,** Western Reserve University, Cleveland, Ohio. Miss Kline in investigating possible relations of known vitamins to the biochemical mechanism of steroid hormone action.

**The University of Pennsylvania** has received $100,000 from Lit Brothers, Philadelphia department store. A major portion of the gift will be used for the development of the University's new Medical Center. This latest donation brought the total gifts and pledges to the University's development fund to more than $4,000,000.

## Fellowships

**The National Paraplegia Foundation** is offering a limited number of fellowships for research in spinal cord disease and trauma and assiciated complications. These $3,000 fellowships are open for the academic year 1949–1950 to any candidate who has demonstrated a capacity for medical research and has outlined a meritorious program of study. Application forms may be obtained from L. W. Freeman, Chairman, Medical Advisory Committee, National Paraplegia Foundation, Room 457, Hotel La Salle, Chicago 2, and must be submitted not later than *June 1.*

**The Jessie Horton Koessler Fellowship** of the Institute of Medicine of Chicago will be available September 1. Established for the aid of research in biochemistry, physiology, bacteriology, or pathology, the fellowship carries a stipend of $500 a year, with the possibility of renewal for one or two years. Applications must be approved by the head of the department in the fields mentioned or by the director of a research institute or laboratory in Chicago, and must stipulate that the applicant will have adequate facilities for carrying out the proposed research. A letter presenting full information should be sent in quadruplicate to Paul R. Cannon, Committee Chairman, 950 East 59th Street, Chicago 37, not later than *July 1.*

## Colleges and Universities

**Ohio State University College of Dentistry** will hold a post-collegiate assembly April 27–28. Dental progress since World War II will be reviewed by members of the faculty, assisted by Charles Williams, University of Toronto, and Robert Hardy of Tufts Dental College.

**The Massachusetts Institute of Technology** is offering a new graduate program in analytical chemistry. Planned to meet new needs in industry and teaching, the course emphasizes instrumental methods, particularly radiochemical analysis. Assistantships and scholarshpis are available, and information may be obtained from the Head of the Department of Chemistry, Massachusetts Institute of Technology, Cambridge 39.

**The University of Washington** is conducting basic research toward developing a simple method for locating warm levels in extreme low-temperature areas. The investigation, carried on under a $6,000 grant from the U. S. Army Quartermaster General's Office, will be used in locating arctic camps, supply dumps, and air fields. Phil E. Church, head of the department of meteorology and climatology is directing the study, and Robert G. Fleagle, assistant professor, is in charge of the preliminary work.

Vassar College has awarded full-year faculty fellowships to **Edward R. Linner,** associate professor of chemistry, and **Monica Healea,** associate professor of physics. Professor Linner will continue a study of absorption from solution, in England or Sweden, and Professor Healea will do research at Vassar in the field of secondary emission.

## Summer Programs

**Duke University Marine Laboratory,** Beaufort, North Carolina, will open its twelfth summer session on June 14. Courses in marine algae and marine ecology will be offered during the first six weeks and courses in plant ecology and marine invertebrate zoology during the second six weeks. They are open to graduate students and seniors only. Further information may be obtained from the Director of the Laboratory, Box 269, Beaufort, North Carolina.

**The Oak Ridge Institute of Nuclear Studies'** Special Training Division will conduct three basic courses in the techniques of using radioisotopes as tracers, June 6–July 1, July 11–August 5, and August 8–September 2. Thirty-two participants will be accepted for each course, and applications should be mailed prior to *June 1.* Forms and additional information may be obtained from Ralph T. Overman, Chairman, Special Training Division, Oak Ridge Institute of Nuclear Studies, P. O. Box 117, Oak Ridge, Tennessee.

**The University of Michigan's** summer symposium on contemporary physics will be held from June 27 to July 30. Guest lecturers will include: Luis W. Alvarez, University of California—"Recent Experiments in High Energy Physics," June 27–July 9; R. P. Feynman, Cornell University—"Problems in Quantum Electrodynamics," July 11–July 30; Frederick Seitz, Carnegie Institute of Technology—"Special Topics of the Theory of Solids," June 27–July 23; and G. B. B. M. Sutherland, Cambridge University—"Current Developments in the Infrared in the Physical, Chemical, and Biological Fields," July 5–July 30. Further information may be obtained from E. P. Barker, Chairman, Department of Physics, University of Michigan, Ann Arbor.

**The Roscoe B. Jackson Memorial Laboratory,** Bar Harbor, Maine, will hold its regular summer student training program June 22–August 30. Two concurrent 10-week courses in research problems, techniques, and progress in the field of growth or of behavior will be offered. Enrollment is limited to 22 students to be selected on the basis of scholarship and interest in particular fields. Further information may be had by writing to the Chairman, Summer Student Program, Jackson Laboratory, Bar Harbor, Maine. Applications must be in by *April 1.*

## Meetings and Elections

Wilder Graves Penfield, professor of neurology and neurosurgery, McGill University, Montreal, will present the **Edward K. Dunham lectures** for the promotion of medical sciences, at Harvard Medical School, March 28 and 30, and April 1. His subject will be "The Cerebral Cortex of Man." The lectures, to which all interested professional persons are invited, will be held at 5:00 p.m. at the Harvard Medical School Amphitheater, Building D.

A conference on **Mathematical Models in the Social Sciences,** under the joint sponsorship of the Department of Mathematics and the Laboratory of Social Relations of Harvard University, will be held March 28 and 29, at 4 p.m. and 8 p.m., in Emerson Hall, Room D, Harvard University. Speakers will be N. Rashevsky, University of Chicago; John von Neumann, Institute for Advanced Study; and Norbert Wiener, Massachusetts Institute of Technology.

**The American Mathematical Society** will hold its 445th meeting April 1-2, at Duke University, Durham, North Carolina. Sessions will be held in Rooms 114 and 117, Physics Building. Registration headquarters will be in the lobby of the Men's Union, West Campus. Hotel reservations should be made directly with the hotels.

**The Association of Geology Teachers** will hold its ninth annual meeting April 22–23 at Rosenwald Hall, University of Chicago. The program includes the presentation of papers on geological education and field excursions. Nonmembers wishing to attend should communicate with Katherine F. Greacen, Secretary, Department of Geology, Milwaukee-Downer College, Milwaukee.

**Yale University's Silliman Memorial Lectures** are being delivered this year by Ross G. Harrison, director emeritus of the University's Osborn Zoological Laboratory. The subject of the six lectures is "Organization and Development of the Embryo," and the forthcoming four will be held at 4:30 p.m., March 25, 30, and 31, and April 1, in Strathcona Hall on the campus.

The Cardiovascular Study Section of the National Institutes of Health, Public Health Service, will hold a **Symposium on Water and Electrolyte Metabolism in Cardiac Edema** at Hotel Haddon Hall, Atlantic City, on April 30. E. Cowles Andrus, of Baltimore, is chairman of the section. Those who expect to attend should communicate with Eleanor M. K. Darby, Executive Secretary, National Institutes of Health, Bethesda, Maryland.

**The American Institute of Chemical Engineers** will hold its regional meeting May 9–11 at the Mayo Hotel, Tulsa, Oklahoma. The program includes four symposia on primary energy, chemicals from petroleum, oil and gas production, and chemical engineering economics. Additional information may be obtained from Clifton Frye, Research Department, Stanolind Oil and Gas Company, Tulsa.

**David J. Finney,** lecturer on the design and analysis of scientific experiment, University of Oxford, will give three lectures on "Statistical Principles of Biological Assay" at the U. S. Department of Agriculture Graduate School. The lectures, sponsored by the Department of Mathematics and Statistics, will be held June 3, 6, and 7 from 4:00 to 5:30 p.m. in the Jefferson Memorial Auditorium, South Agriculture Building, Washington, D. C. The public is invited.

**The Genetical Society of Great Britain** will hold its 100th meeting June 30–July 1, in Cambridge. Guest speakers will review the early days of genetics and demonstrate genetical work now in progress in Great Britain. All geneticists are cordially invited. Details may be obtained from the secretaries, G. Pontecorvo, Department of Genetics, The University, Glasgow, W. 2., and H. G. Callan, Institute of Animal Genetics, The University, Edinburgh 9.

**Mining Engineers in San Francisco.** In celebration of the centenary of the Gold Rush, mining engineers, economic geologists, and metallurgists assembled in San Francisco February 13–17 for the 168th general meeting of the American Institute of Mining and Metallurgical Engineers. The Society of Economic Geologists met jointly with the Institute. Members of the U. S. Section of the Pan-American Institute of Mining Engineering and Geology gathered informally for luncheon, but arranged no special program.

William E. Wrather, treasurer of the AAAS, who has served the Institute as president during the past year and acted as presiding officer at general sessions and business and directors' meetings, relinquished the post to L. E. Young, incoming president for 1949. Howard A. Meyerhoff, administrative secretary of the AAAS, took office as chairman of the Industrial Minerals Division for the year 1949.

A record crowd divided its attention among 60 technical sessions, divisional luncheons, and social functions, and heard speeches by Governor Earl Warren at the All-Institute luncheon, and by Robert Gordon Sproul, president of the University of California, at the annual banquet. Noteworthy among the sessions were symposia on national mineral policy and atomic mineral deposits. None of the technological developments which make the mining and metallurgical professions dynamic was neglected, but programs and papers dealing with economics in the Industrial Minerals, Petroleum, and Coal Divisions gave clear indication that even the experts recognize that technology is not enough, and that the engineer must broaden his horizon to embrace the remotest economic factors which affect his operations.

The engineers honored distinguished colleagues, awarding medals to Harry Carothers Wiess (posthumously), Stanly A. Easton, William Wraith, and Eugene McAuliffe. Prizes and other awards for research went to M. Tenenbaum, E. A. Gulbransen, and J. W. Hickman (posthumously), John D. Saussaman, and Robert L. Hoss. Little time was allotted to field Activities, but the visiting engineers were given a courtesy flight over the San Francisco Bay area by Pan American Airways.

Plans for 1949 were laid in business sessions, and a regional meeting was

announced for September in Columbus, Ohio. The next annual meeting will be held at New York, in February, 1950.

HOWARD A. MEYERHOFF

## Deaths

**W. Reid Blair,** 74, zoologist, died March 1 in New York City of a heart attack. Dr. Blair retired in 1940 as director of the New York Zoological Society's Bronx Zoo, where he had served for 32 years.

**Theodore M. Focke,** 78, former dean of Case Institute of Technology, died March 2 in Cleveland, Ohio. A Case faculty member for nearly 52 years, Dr. Focke was appointed professor of mathematics in 1908 and was dean of the Institute from 1918 until his retirement in 1944.

**Leon Hastings Cornwall,** 62, neurologist and member of the faculty at the College of Physicians and Surgeons, Columbia University, died at White Plains, New York on March 4.

**Franklin S. Newell,** 77, gynocologist and professor emeritus of clinical obstetrics at Harvard Medical School, died in Boston March 4.

**Edwin H. Colpitts,** 77, electrical engineer, died March 6 in East Orange, New Jersey. Dr. Colpitts played a major part in the development of telephone and radio communication and in the establishment of trans-Atlantic telephone service.

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New Yorkers were flocking to the beach just about three months early when the New York Botanical Garden's exhibit, **"Plants Beside the Sea,"** opened March 21, at the International Flower Show, Grand Central Palace. Showing the characteristic northeast coastal plants of North America, which have adapted themselves to wind, salt, and sand, the Botanical Garden's presentation will include a miniature beach, replete with seaside cottage, cliffs, lapping waves, and sandy shoreline, as well as grass, heather, pines, and other plants found near the ocean. This month's issue of the Botanical Garden's *Journal* carries an article listing more than 100 seacoast flowers —"Wild Flowers of Our Coastal Lands," by E. J. Alexander. Single copies may be ordered for 15 cents from the Botanical Garden, Bronx Park, New York City 58.

**The American Museum of Atomic Energy** was opened to the public on March 19, when the gates to the city of Oak Ridge were removed. The exhibit is the first step in a broad program of public education in the field of atomic energy taken by the Oak Ridge Institute of Nuclear Studies, which heretofore has confined its activities to highly specialized research and training in the nuclear sciences. The "Man and the Atom" show, originally presented at the New York City Golden Jubilee last summer, forms the nucleus of the Museum. A staff is being assembled under the direction of David Lloyd DeJarnette, who has taken a year's leave of absence from his post as curator of the Alabama Museum of Natural History for the purpose. Admission fees will be used to expand and improve the exhibits.

Scholars from overseas who visit Boston are invited to visit the **American Academy of Arts and Sciences,** 28 Newbury Street, Boston 16, Massachusetts. The staff of the Academy will be glad to arrange travel and hotel accommodations and introductions to scholars and institutions in the Boston district. Mail and telephone calls may also be received at the Academy.

**Harvard University's Peabody Museum** has announced the discovery of an engraved pebble believed to be the finest example of Aurignacian engraving thus far discovered by archaeologists. The pebble was found last summer during excavations at La Colombiere rock-shelter, above the Ain River in eastern France, in an occupation layer immediately overlying the terrace that contained the relics of the Upper Aurignacian period, which places its age at 20,000 to 25,000 years. It is limestone, about the size of a large potato, and covered on both upper and lower surfaces with a number of engravings superimposed one over the other. Archaeologists have outlined a rhinoceros, horse, ibex, bison, and deer, as well as other partially drawn animals. They believe that the stone was used over and over again to insure good hunting. Excavations leading to the discovery were made by H... lam Movius, Jr., curator of pal... lithic archaeology, and Kirk Bry... professor of physiography, of H... vard University, with the assistan... of Louis Dupree and Carleton P... pont of Harvard, and S. Shel... Judson, of the University of W... consin.

**Water-tight tile walls** are possi... through the use of a dense w... graded mortar applied in well-fi... joints, the Office of Technical Se... ices of the Department of Comme... reports. University of Minnesota ... searchers, who made the study u... the OTS industrial program, expo... sample tile wall sections to simula... conditions of wind and rain to f... the best ways to prevent wall leak... during prolonged exposure. Th... found that the bond between tile a... mortar has key significance in t... water-resistance of a wall, and ... more important than the kind of t... used. A copy of the report, PB94... *Water permeability of structural cl... tile facing walls*, may be ordered fr... the Office of Technical Services, D... partment of Commerce, Washing... 25, D. C. The price is $1.00 and che... or money orders should be payable ... the Treasurer of the United States.

## Make Plans for—

**Michigan Academy of Scien... Arts, and Letters,** March 31–April ... Wayne University, Detroit.

**2nd Symposium on Plasticity** sponsored by the Navy Department's Bureau of Ships and Office of Na... Research, April 4–6, Brown Univ... sity, Providence, Rhode Island.

**Meeting of the Animal Disea... Research Workers in The Southe... States,** April 7–8, Alabama Polyte... nic Institute, Auburn.

**Crystallographic Society of Amer... ica,** fourth annual spring meeti... April 7–9, University of Michig... Ann Arbor.

**Eastern Psychological Associa... tion,** annual meeting, April 8-... Springfield, Massachusetts.

**Medical Library Association,** a... nual meeting, April 10–14, Galvest... Texas.